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THE UNIVERSITY OF ALBERTA

WATER SORPTION STUDIES OF HEAT DENATURED WHEY PROTEINS

by

© ROBERT I.W. GREIG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled 'WATER SORPTION STUDIES OF HEAT DENATURED WHEY PROTEINS' submitted by ROBERT I.W. GREIG in partial fulfilment of the requirements for the degree of Master of Science.

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(Supervisor)

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Date ....September 22,....1978

ABSTRACT

The cause of loss in water holding capacity (WHC) after drying of denatured whey protein, was investigated using a water holding capacity test and water sorption isotherms. Various methods of drying have caused a 20-80% loss in WHC; freeze drying having the least significant effect.

The bulk density of the powders as influenced by powder composition and drying method, appeared to be the major factor affecting the WHC.

Adsorption-desorption isotherms of the heated protein powders were studied to determine the BET monolayer moisture content, BET heat of sorption and the porosity. The drying method affected the monolayer moisture content and the net heat of adsorption. There was no relationship between powder porosity and the monolayer value of the powders. The outer surface of the powders (as revealed by scanning electron microscopy) seemed to influence the monolayer moisture content. Changes in the number and activity of water sorption sites are the probable cause of variation between monolayer values.

Water sorption isotherms could not be used to predict the WHC of denatured whey protein powders. Even though there was no conclusive relationship between monolayer moisture content (m_o) and WHC, a general trend was observed. As the m_o value decreased (in relation to the severity of the drying process) the WHC decreased.

The loss in WHC of denatured whey protein powders is most likely due to physical changes during dehydration. These include changes in the surface and internal structures of the powders; changes in the number of active sites available for water sorption and reduction in the surface area available for water sorption.

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CHAPTER 1

INTRODUCTION

1.1 The Problem of Whey

Whey is the liquid portion of milk obtained during the manufacture of cheese or industrial casein. There are two major types of whey - acid whey from cottage cheese, bakers cheese and acid casein manufacture, and sweet whey from cheddar, Swiss and American cheeses and rennet casein.

Whey is an excellent source of nutrients but increased cheese production and underutilisation of whey has led to what could become a serious problem in the near future. Whey has a very high biological oxygen demand (approximately 35,000 mg/l) and, if not utilised, poses a very serious pollution threat in inland rivers and lakes. As well as being a serious pollutant, a valuable source of nutrients is being lost.

According to various sources, seven billion kilograms of whey will be produced this year in the U.S.A., one of the biggest producers of whey in the world. If we assume that this whey has a protein content of approximately 0.7% (Gillies, 1974), 49 million kilograms of high quality protein are available from this source. At the present time, utilisation of whey accounts for about 50% of the whey produced, so there is an annual net loss of 25 million kilograms of protein from cheese whey in the U.S.A. alone. The scope of the problem is the same in Canada and Australia although on a smaller scale.

Because of more stringent pollution regulations, an increased emphasis has been placed on the recovery of nutrients from whey.

The traditional methods of spray drying, drum drying and heat-acid coagulation of whey protein have been re-investigated and many new (and sometimes bizarre) methods have been proposed for the recovery of nutrients from whey. The recovery of whey protein has received considerable attention during the last 25 years and many methods have been developed to produce an acceptable whey protein concentrate. Each method produces a slightly different product and they all seek to retain (or improve) some of the important functional properties of the whey protein. These include whippability, emulsifying capacity, gelling ability, solubility and water holding capacity.

The maintenance of some (or all) of these functional properties during the manufacture of a whey protein preparation is extremely important. Smith (1976) states; *The physical properties of milk proteins are the main reasons for their use in the food industry at the present time, few applications rely solely on nutritional attributes alone.*

One method of producing whey protein, is the traditional method of heat-acid coagulation. The method has some inherent advantages in that it requires minimal capital outlay, the whey protein is easy to produce and the method is suitable for use in dairies that have small or seasonal whey production. The high water holding capacity of the wet curd (in the range 660-700 g water/100 g dry solids) is another distinct advantage of this process. There are a number of problems with the method and two of the more serious are:-

(i) After precipitation of the protein, the supernatant is still a potential pollutant as it contains most of the lactose and about 20% of the protein nitrogen that is not heat precipitated under the process conditions.

(ii) Upon dehydration, the whey protein powder obtained from the process loses a significant amount of its water holding capacity.

The problem of loss of rehydratability of the curd has been referred to by Gillies (1974): *If high protein whey preparations are made so that protein becomes insoluble during concentration and drying, then in a reconstituted aqueous mixture the protein will settle rapidly. In coagulating, whey protein absorbs and binds large amounts of water. A coagulated whey protein gel may contain 70% water but still appear dry and crumbly. If the gel is dehydrated, the water cannot be reincorporated. Dehydration is irreversible.*

This statement is not entirely true. Work in New Zealand (Knightbridge and Goldman, 1975; Short *et al.*, 1978) and preliminary work leading to this thesis, has shown that rehydration of denatured whey protein curd can be accomplished but at a level of 20-80% of the original curd.

1.2 Definition of Terms

To investigate why denatured whey protein powders will not readsorb all of the water lost during dehydration of the curd, a study of the water holding capacity (WHC) and water binding capacity (WBC) of the powders was initiated. Because many terms such as WHC and WBC are being used in the literature without definition and, what is worse, different terminology is being used to explain the same characteristic; the following are defined for the purpose of this work:-

Whey Protein: this term is used to loosely describe, as a group, all of the proteins naturally found in whey.

Heat-Denatured Whey Protein (or Denatured Whey Protein): This is the material observed after precipitation and separation of all heat coagulable whey proteins. The term used describes the mixture of several heat denatured proteins and should not be misconstrued as being a single component. In the literature, it is occasionally referred to as "traditional lactalbumin" and the terms are used synonymously in this work.

Water Binding Capacity (WBC): the capacity of the material to hold a certain quantity of water at the active sites on the surface of the substrate. This quantity is envisaged as a monolayer of water assuming constant coverage of the substrate active sites at constant temperature. The determination of the extent of the monolayer coverage using the method of Brunauer *et al.* (1938) was used as a measure of this quantity.

Water Holding Capacity (WHC) = Water Absorption Capacity: the capacity of the material to hold a certain quantity of water in the capillaries and voids of the substrate after surface adsorption. For the purpose of this definition, most of this water is considered to be "free", i.e. it is not chemically or physically bound to the active sites, and has the following properties:-

(i) it is able to exert vapor pressure;

(ii) it can assume an ordered structure when the temperature is lowered (i.e. it can freeze);

(iii) it is able to act as a solvent.

The WBC and WHC of various proteins are referred to in the literature as functional properties of the proteins concerned. Various methods are described by which these functional properties of WHC and

and WBC are altered so as to improve the "functionality" of the protein. To the author's knowledge, no-one has adequately attempted to explain, in physical terms, just what WHC and WBC are, although Ryan (1977) has published an excellent review on some of the factors that may be involved in determining the functional properties of proteins. To quote Ryan (1977): *Criteria for evaluating what is and what is not an improvement in a food protein are at present poorly defined.....* At the moment, functional properties such as WHC and WBC are being altered without the basic knowledge of what is happening in the food protein to cause such a change. In other words, it is a "hit or miss" approach when the functionality of food proteins is concerned.

This work has attempted to identify some of the physical changes that have occurred during the dehydration and rehydration of denatured whey protein powder.

1.3 Research Objectives

The prime objective of this work was the study of the irreversible loss in water holding capacity of denatured whey protein powders. Various workers have stated that this loss occurs but, to the author's knowledge, no attempt has been made to explain why it occurs. To determine why these powders will not rehydrate to the original WHC of the fresh curd, the following variables were examined.

(a) The method of dehydration of the powders

Preliminary observations and the work of Knightbridge and Goldman (1975) and Short *et al.* (1978) showed that the loss in water holding capacity seemed to vary with the drying method. Five different drying methods (freeze drying, spray drying, air drying, vacuum drying and drum drying) were investigated to determine if this was a general effect and to what

extent each drying method would affect the WHC.

(b) The composition of the powders

The effect of composition on WHC was studied in an attempt to identify some possible effect of interactions (protein-protein, protein-lipid, protein-lactose, protein-ion) on WHC.

(c) Sorption characteristics

Water sorption isotherms of each powder were produced. An analysis of these isotherms by the method of Brunauer *et al.* (1938) enabled the calculation of the monolayer value, the heat of sorption and the porosity of each powder. Two important questions were to be answered in this study:-

(i) could the amount of "bound" water (as determined by the method of Brunauer *et al.*, 1938) be related to the WHC of a powder?

(ii) would the drying method affect the BET monolayer value of the powder?

(d) Physical characteristics of the powders

With a large number of inorganic substrates and some foodstuffs, the porosity determines the sorption characteristics. At the same time, the surface geometry of a substrate will influence the rate or extent to which water can penetrate into the substrate. A calculation of internal porosity from the sorption isotherms and comparison of external surface by scanning electron microscopy (SEM) was undertaken to determine if these properties had any effect on the WHC of denatured whey protein powders. The SEM of the powder surface was performed at the Food Research Institute, Ottawa, by Dr. M. Kalab. The author is indebted to Dr. Kalab for the valuable information on the

surface features of each powder provided.

Another important physical parameter in any dried material is the bulk density. The bulk densities of all powders were determined to see if this variable could also be used to explain differences in WHC.

From the manufacturer's point of view, the storage of dried products is of prime importance. To conclude this study, samples of a freeze dried powder were stored under different temperature conditions for up to 200 days to see if storage time and/or temperature would alter the WHC of the powders. The degree to which the powders bind and absorb water and the resulting changes in WHC as a result of dehydration or storage conditions, will determine the acceptance of the powders for use in food products.

CHAPTER 2

LITERATURE REVIEW

2.1 Whey Composition

When milk is processed to make cheese or casein, liquid whey is separated from the curd. Liquid whey contains almost all of the water soluble minerals and vitamins, most of the lactose and about 20% of the total milk proteins. Typically, whey contains approximately 50% of the solids present in milk (Forsum, 1975; Smith, 1976). A typical cheese whey would have 6-6.5% solids of which lactose is about 4.5% and proteins 0.6-0.7%; the balance being variable quantities of fats, minerals and minor constituents. Table 1 summarizes an average composition of cheese wheys. As noted in this table, wheys from different sources can have different compositions. Usually cottage cheese whey has higher protein and mineral contents and lower lactose content than whey from other sources.

Table 1: Average Composition of Cheese Wheys

Component	Cheddar Whey (%)	Cottage Whey (%)
Protein	0.60	0.7
Non-protein nitrogen	0.20	-
Fat	0.04	0.05-0.10
Lactose	4.6-4.8	4.3-4.5
Ash	0.60	0.6-0.75
Total Solids	~6.6	6.1-6.5

2.1.1 Whey Proteins

The protein composition of whey has been described in great detail by McKenzie (1971) and an extensive update on the chemistry of whey proteins has been provided by Lyster (1972). Whey contains a number of protein fractions of which the predominant species (making up almost 50% of the total whey protein) is β-lactoglobulin. Serum albumin, α-lactalbumin, proteose-peptone fractions and immunoglobulins make up the remaining 50%. Table 2, a composite table prepared from a number of literature sources, summarizes the components of whey protein and their important properties.

(a) β-lactoglobulin

The most abundant of the whey proteins is β-lactoglobulin. Four variants of β-lactoglobulin have been reported (A,B,C,D) and the amino acid differences of each variant determined (McKenzie, 1971). It was originally thought that β-lactoglobulin was a monomer of molecular weight 36000 daltons, but Fraenkel-Conrat (1954) showed that the protein existed in its native state as a dimer. The dimeric form of β-lactoglobulin is stable in the pH range 3.6-6.0 (McKenzie, 1971). In solution, there is a dynamic equilibrium between the dimeric and monomeric forms. At pH 5.2, the dimer is weakly dissociated to the monomer. As the pH lowers (or raises above 5.2) the equilibrium dissociation constant increases and more of the monomeric form is produced. Below pH 3.5, the rate of conversion to the monomeric form is quite appreciable (McKenzie, 1971).

The amino acid composition of β-lactoglobulin has been well documented and is shown in table 3. The sequence of the amino acids is not fully known but a partial sequence has been assigned to

Table 2: Some Important Properties of Whey Protein

Whey Protein Fraction	Weight % of Whey Protein (a)	pI (b)	Sedimentation Constant ($S_{20,w}$) (c)	Molecular Weight (d)	Components (d)
α-lactalbumin	19.7	5.1	1.75	14,437	Variants A and B
β-lactoglobulin	43.7	5.3	2.7	36,000	A, A_{Dr}, B B_{Dr}, C, D
Serum Albumin	4.7	4.7	4.0	69,000	—
Immunoglobulins	13.0	—	6.3-19.0	$1.5x10^4$-$1.0x10^6$	—
Proteose-peptone fraction	18.9	3.3-3.7	0.8-4.0	41,000-$2.0x10^5$	multiple including glycoproteins

(a) Smith, 1976

(b) Isoelectric pH (Rose *et al.*, 1969)

(c) Sedimentation constant in Svedberg Units (Rose *et al.*, 1969)

(d) Rose *et al.* (1969)

Table 3: Amino Acid Composition of Bovine α Lactalbumin B and Bovine β Lactoglobulin

Amino Acid	α Lactalbumin B			β Lactoglobulin			
	Residues per M.W. 15,500	Residues per M.W. 15,000	Residues per M.W. 14716	Variant A	Variant B	Variant C	Variant D
Asp	22	23	21	16	15	15	16
Thr	7	7	7	8	8	8	8
Ser	7	7	7	7	7	7	7
Glu	14	13-14	13	25	25	24	25
Pro	2	3—4	2	8	8	8	8
Gly	7	6	6	3	4	4	4
Ala	4	3—4	3	14	15	15	15
Cystine	4	—	4	2	2	2	2
Cysteine	—	—	—	1	1	1	1
Val	6	6	6	10	9	9	9
Met	1	1	1	4	4	4	4
Ile	8	8	8	10	10	10	10
Leu	14	14	13	22	22	22	22
Tyr	5	4	4	4	4	4	4
Phe	4	14	4	4	4	4	4
Lys	12	13	12	15	15	15	15
His	3	3	3	2	2	3	2
NH_3	15	—	—	15	15	14	15
Arg	1	1	1	3	3	3	3
Trp	4	—	4	2	2	2	2

From Smith (1976)

approximately 50% of the amino acids (McKenzie, 1971). β-lactoglobulin is known to contain free sulphydryl groups in the form of cysteine residues (two per dimer). These groups are thought to be involved in the "cooked flavor" that develops in heated milk (Tummerman and Webb, 1965) and in the coagulation of β-lactoglobulin during heat denaturation (Harper and Hall, 1976). Considerable controversy still surrounds the arrangement and position of the disulphide bonds and the SH group, and Smith (1976) has illustrated possible arrangements of the above as postulated by McKenzie and Ralston (1971) and Mainferme *et al.* (1971).

A biological function for β-lactoglobulin has not yet been established.

(b) α-lactalbumin

Gordon (1971) and Lyster (1972) have reviewed, in great detail, the chemistry of α-lactalbumin. Table 3, which summarizes the amino acid composition of α-lactalbumin, reflects the different techniques used by the researchers. Gordon and Ziegler (1955) calculated a molecular weight of 15,500 whereas Brew *et al.* (1967) calculated a molecular weight of 14,716 (119 amino acid residues). Brew *et al.* (1970) published the complete amino acid sequence based on 123 residues. Subsequent ultracentrifugal analysis by Gordon (1971) has shown that the protein has a molecular weight in the region of 16,000 daltons.

An interesting aspect of α-lactalbumin is its apparent heterogeneity (Lyster, 1972). In pH 3.3 lactate buffer, α-lactalbumin shows two electrophoretic peaks. The aggregation of α-lactalbumin below its isoelectric pH parallels a "denaturation-like" process where certain groups are made available for intermolecular interaction.

This conformational change is temperature and pH dependent. Changes in the side chain (as shown by shifts in the absorption spectrum in the region 270-300 nm) and not conformational changes in the backbone of the peptide chain could be responsible for this interaction. It is of interest to note that Kronman (1968) has shown no significant conformational differences between native α-lactalbumin and acid-denatured lactalbumin using circular dichroism measurements in the range 185-300 nm. The effect of temperation on heat denaturation of α-lactalbumin and β-lactoglobulin will be discussed in section 2.2.1.

Unlike β-lactoglobulin, α-lactalbumin has an extremely important biological function — it is a component of the enzyme known as lactose synthetase. This enzyme is found in soluble form in bovine milk. It consists of two parts, A and B; B being α-lactalbumin (Lyster, 1972). Lactose synthetase catalyses the linkage between glucose and galactose to form lactose.

The discovery of the biological function of lactalbumin has created renewed interest in the chemical structure of this protein.

(c) Proteose-Peptone fraction

Associated with the whey proteins, is a group of proteins loosely called the "proteose-peptones". This is a large heterogeneous group of proteins consisting mainly of glycoproteins and phosphoproteins. Smith (1976) describes this group as *...that portion of the protein fraction (of whey) not precipitated by heating at 95°-100°C for 20 minutes and subsequent acidification to pH 4.7... but precipitated by 12% W/V trichloroacetic acid.*

Larson and Rolleri (1955) found three separate peaks at pH 8.6 using moving boundary electrophoresis. These peaks were designated as

components 3, 5 and 8 in increasing order of electrophoretic mobility. Ng and Brunner (1967) and Kolar and Brunner (1968) were able to isolate fractions 3, 5 and 8 from skim milk and Kolar (1967) was able to further fractionate the proteose-peptone components into four distinct fractions. Some of the important characteristics of the proteose-peptones are listed in table 2.

(d) Immunoglobulins

The immunoglobulins are a heterogeneous group of high molecular weight glycoproteins which include some molecules with antibody activity. Three major classes of immunoglobulins have been found in cows' milk and characterised; IgG, IgA and IgM (Lyster 1972; Smith 1976). In general, all of the immunoglobulins appear to be either monomers or polymers. The main polymer sub-units are two heavy and two light polypeptide chains with molecular weights of 60000 and 20000 daltons respectively (Lyster, 1972). This four polypeptide chain sub-unit of the immunoglobulins is held together by disulphide bridges (Rose *et al.*, 1969). Lyster (1972) states that IgA is often found as a dimer (consisting of two of the tetramer subunits), IgG is usually a monomer and IgM is usually a pentamer (consisting of five of the tetramer units). Some of the primary characteristics of the immunoglobulins as a class, are given in table 2.

2.1.2 Lactose

Lactose is the major constituent of the whey solids making up approximately 75% of the total solids.

In solution, lactose exists in two forms — α and β. When either of these forms is dissolved in water, there is a gradual shift from one form to the other until an equilibrium is established. This

effect is known as mutarotation. At 20°C, the equilibrium ratio of β to α is always 1.68 (i.e. 37.3% of the lactose is in the α form and 62.7% is in the β form) and the specific rotation is always 55.3° (Nickerson, 1970). The equilibrium ratio is temperature dependent. As the temperature rises, the proportion of α-lactose increases i.e. the equilibrium ratio, β/α, will decrease as the temperature increases.

In the solid state, lactose normally exists as one of three forms:-

(i) α-lactose hydrate; this is the monohydrate form of lactose which crystallises from a supersaturated lactose solution at a temperature of 93.5°C or less.

(ii) β-lactose anhydride; crystallises from a supersaturated solution above 93.5°C; this form is sweeter and more soluble than the α-hydrate.

(iii) lactose glass. When a solution of lactose is dried quickly, crystallisation of lactose is inhibited and a non-crystalline glass is formed. This glass contains an equilibrium mixture of α and β-lactose. The amount of each will vary according to the equilibrium ratio of the solution prior to drying.

The most common form of lactose encountered in spray dried, drum dried or freeze dried milk products is lactose glass. Lactose glass is extremely hygroscopic and will absorb moisture until a point is reached (approximately 8% moisture on a dry basis) where α-hydrate crystals will form (Berlin *et al.*, 1968). The formation of lactose glass can be a serious problem when drying dairy products containing large amounts of lactose.

2.1.3 Minor Constituents

Calcium, phosphorus, iron, sodium, potassium and magnesium ions are the most abundant metal ions found in whey. Potassium accounts for about 45% of the total metal ions; calcium, phosphorus and sodium make up 16%, 20% and 15% of the total ions respectively; the other 4-5% is iron and other trace metals (Corbin and Whittier, 1965; Smith, 1976).

The salts of importance are considered to be the chlorides, phosphates and citrates of potassium, sodium, magnesium and calcium. It is thought that potassium, sodium and chlorine are entirely in solution (and ionised) whereas the phosphates, calcium, magnesium and citric acid are partly ionised and partly in colloidal suspension (Corbin and Whittier, 1965).

2.2 Effects of Heat on Whey

2.2.I Heat Denaturation of Whey Proteins

The heat-acid coagulation process for the production of whey protein curd is based on the heat sensitivity of whey proteins. Above 60°C, whey proteins begin a gradual coagulation. The immunoglobulins are the most heat sensitive, being completely denatured at 70°C (Larson and Rolleri, 1955). The most heat stable protein is α-lactalbumin with serum albumin and β-lactoglobulin being intermediate. Serum albumin is thought to be completely denatured at 64°C, β-lactoglobulin 79-80°C and α-lactalbumin 96-114°C (Larson and Rolleri, 1955; Itoh *et al.*, 1976).

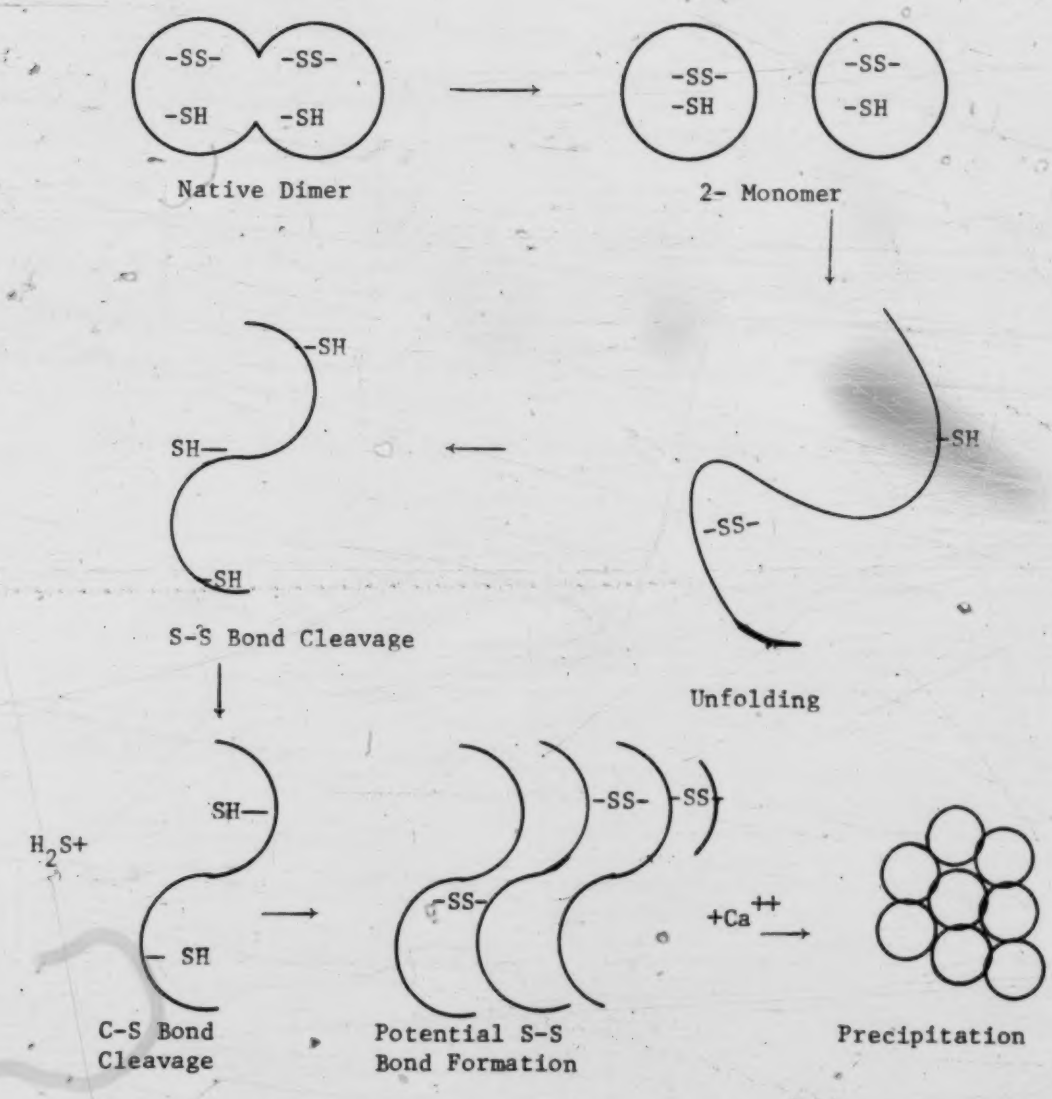
The process of denaturation involves changes in the secondary and tertiary structure of the protein — it does not involve hydrolysis of the covalent bonds present in the primary structure. The denaturation

process can be reversible (e.g. reactivation of the phosphatase enzyme after milk pasteurisation) or, usually, irreversible as in the case of prolonged heating or the addition of chemicals. Heat is probably the most important denaturing agent and when a protein molecule is heat denatured, it will not return to its native conformation. Because of this alteration in protein structure on denaturation, many changes (including loss in solubility) occur in the properties of the protein (O'Sullivan, 1971). In general though, the denaturation of whey proteins is due to an unfolding of their globular structure to a less compact random configuration (Morr, 1975).

Much of the early research on the denaturation of specific whey proteins was undertaken using zonal electrophoresis and immuno-electrophoresis. Steim (1965) first used differential thermal analysis (DTA) in a study of the denaturation of proteins in solution. Itoh *et al.* (1976) used DTA to determine the effect of salt and sugar solutions on whey protein denaturation. This work showed an affect of pH on denaturation temperature of β-lactoglobulin; at pH 5 the denaturation temperature was 81.5°C and this decreased to 66.5°C at pH 9. Heating β-lactoglobulin in the region of pH 9 increases the activity of the sulphydryl groups thus making the protein more susceptible to denaturation (McKenzie, 1971).

As with other proteins, the separation of proteins from whey by heat involves both denaturation and aggregation (Tumerman and Webb, 1965; Lyster, 1972). Figure 1 is a diagrammatic representation of the changes that may possibly occur during denaturation and heat degradation of β-lactoglobulin. The exposure of the sulphydryl groups, due to unfolding, changes the nature of the protein so that

Figure 1: Diagrammatic Representation of Heat Coagulation of β Lactoglobulin



from Harper and Hall, 1976.

aggregation occurs. There appear to be two steps in the aggregation of β-lactoglobulin following denaturation. The first step is an aggregation thought to involve disulphide linkages but not calcium. These aggregates form complexes up to 200000 daltons molecular weight and are not able to be sedimented at x1000 g. The second aggregation step involves the presence of calcium ions to form precipitable particles that will sediment at x1000 g (Morr, 1975).

The largest component of whey protein, β-lactoglobulin, dominates the course of heat denaturation of proteins from whey. The heat denaturation curve for whey proteins closely follows the heat denaturation curve for β-lactoglobulin (Tumerman and Webb, 1965). The reaction is pH dependent, it has a high activation energy (> 48 kcal/mole) and it is strongly influenced by the ionic composition of the solution. The effects of solution components on β-lactoglobulin denaturation will be discussed in section 2.2.2.

Both the denaturation and aggregation of β-lactoglobulin follow second order kinetics but the denaturation of α-lactalbumin follows first order kinetics. It is thought that the denaturation of α-lactalbumin is initiated by the action of the exposed thiol groups from β-lactoglobulin on the disulphide bridges of the α-lactalbumin molecule (Lyster, 1972).

2.2.2 Effect of Ions and Lactose on Whey Protein Denaturation

From about 1940 onward, considerable research attention was directed towards the effect of heat on metal ions and lactose in milk and the resultant effect on the stability of milk proteins.

Van Kreveld (1949) showed that the soluble calcium and phosphorus content of milk was reduced by heating. Tumerman and Webb (1965) report that about 35% of the soluble calcium in milk is ionised and the rest is complexed to citrate and phosphate. Many researchers have shown that heating reduces the total soluble and ionic calcium con-

centrations in milk and whey (Webb and Johnson, 1965).

It is thought that this reduction is caused by a conversion of soluble calcium phosphate to the colloidal state. Upon heating, there is a recrystallisation of calcium phosphate to hydroxyapatite (Evenhuis and de Vries, 1956). This process begins at a temperature of 60°C and increases rapidly as the temperature is increased. According to Evenhuis and de Vries (1956) approximately 30% of the calcium phosphate in rennet whey is precipitated at 100°C. In whey, the calcium phosphate can be readily precipitated with the denatured proteins depending on the pH. The calcium phosphates are mostly soluble in the pH range 4-5 (Harwalkar and Emmons, 1969) and processes that induce precipitation under these pH conditions have low ash contents in the denatured whey protein curd.

An effect common to both milk and whey systems is the increase in acidity during heating. Miller and Sommer (1940) showed that the pH of skim milk decreased approximately 0.1 pH unit for every 10°C rise in temperature and that this change was enhanced by calcium. Unpublished results by the author of this thesis have shown the same increase in acidity (0.1 pH unit/10°C) for neutralised cottage cheese whey (pH 6.5) but calcium caused no significant change in pH as heating progressed.

The increase in acidity is thought to be due to a number of factors which include:-

(i) thermal decomposition of lactose to formic and lactic acids;

(ii) caramelisation of lactose catalysed by the phosphates and citrates naturally present in whey;

(iii) crystallisation of colloidal dicalcium phosphate to hydroxy apatite with subsequent decomposition to more acidic phosphates above 60°C.

Lactose-protein interaction (Tumerman and Webb 1965) is another important source of acidity. The drop in pH can be attributed to:-

(i) an initial increase in acidity due to the binding of lactose to the basic amino acids; it is thought that this bond occurs between the glucose moiety of lactose and the ε-amino group of lysine;

(ii) breakdown of the unstable protein-sugar complex to various acidic decomposition products; it is thought that the basic amino groups (in particular the ε-amino groups of the lysine in whey protein) act as alkaline catalysts in the breakdown of lactose.

In 1965, Webb and Johnson stated... *the development of a unified theory of heat coagulation has yet to be realised, but very substantial progress has been made...* Thirteen years later this statement still holds true. Much of the earlier work on whey protein was concerned with the denaturation of whey proteins in milk and the effect that casein and calcium had on this denaturation. It is only within the last 10 years that a substantial research effort has been placed on denaturation of individual whey proteins in whey systems and the effect that components, such as metal ions and lactose, have on the course of denaturation.

Several workers have shown the dependence of milk and whey protein precipitation on ionic strength of the solution. In the presence of calcium ions, the precipitation and aggregation of β-lactoglobulin is

extremely pH dependent. It is thought that calcium interacts with negatively charged carboxyl sites on the protein molecule, thus reducing the net charge on the protein and, ultimately, rendering it insoluble. Between 1956 and 1958, Zittle and co-workers (Tumerman and Webb, 1965) very thoroughly showed the relationship between pH and calcium ion-protein interaction.

As the protein solution becomes more alkaline, greater quantities of calcium are required to precipitate the protein. As the pH is reduced, there is a decrease in the net negative charge on the molecule (due to protonation of some of the carboxyl groups) and precipitation will occur with decreasing amounts of calcium ion present.

Although the calcium ion concentration in milk is sufficient to precipitate β-lactoglobulin during heating, the denatured serum proteins do not coagulate to any extent. This is generally thought to be due to a heat induced link between β-lactoglobulin and casein (Morr and Josephson, 1968). The importance of this interaction in heated whey systems is not known although it would most likely be negligible because of the small amount of casein present in whey.

Unfortunately, with most research on heat denaturation of whey proteins, there is virtually no information on possible interactions between the various components that make up "whey protein" as defined in this work. Most research work seems to be centered on whey protein as a whole or on β-lactoglobulin, presumably because it is the largest single component in whey protein and is thought to relate to the heat denaturation curve for the total whey proteins. The occurrence of interactions among the components making up whey protein has not been adequately explored.

Nielsen *et al.* (1973) showed that there was a complex relationship between pH, time, temperature and total solids on the extent of whey protein denaturation. Of importance is their finding that these variables do not necessarily effect denaturation of whey proteins in different whey systems in the same manner. As an example, with cottage cheese whey, the most important variable affecting protein denaturation was pH; time of heating was the most important variable for colby cheese whey.

The effects of several variables on the course of heat denaturation of whey proteins can be summarised as follows:-

(i) pH: Guy *et al.* (1967) showed that maximum protein stability occurred at pH 3.4. This is in apparent conflict with Nielsen *et al.* (1973) who showed that minimum protein denaturation occurred in the region pH 6-7.

(ii) Calcium: Heat denatured whey proteins in heated whey systems are prone to physical aggregation due to calcium ion initiated interaction (Morr and Josephson, 1968). Neither the addition of calcium nor increased heating temperatures increased the yield of protein precipitation (60-70%). Addition of calcium reduced the heating time required to achieve maximum precipitation (Hidalgo and Gamper, 1977).

(iii) Total Solids: Whey proteins were least susceptible to protein denaturation at 20% total solids (Guy *et al.*, 1967). At 60% total solids (pH 6.5, 75°C, 15.5 minutes) all of the whey proteins including the heat labile immunoglobulins were stabilised against heat denaturation

(Nielsen *et al.* 1973).

(iv) Temperature: At temperatures less than 65°C, there was virtually no protein denaturation (Larson and Rolleri, 1955; Nielsen *et al.*, 1973). Increasing the temperature above 95°C did not increase the yield of denatured protein but the time required to achieve maximum protein precipitation was decreased (Hidalgo and Gamper, 1977).

2.3 Industrial Production of Denatured Whey Protein Concentrates

2.3.1 The Heat-Acid Coagulation Process

Between 1940 and 1960, considerable research was directed towards the heat-acid coagulation process. This method seemed to have met with disfavor when attention shifted to producing undenatured whey protein concentrates. The trend now appears to be shifting slowly back to the investigation and production of heat-acid coagulated whey protein.

In general, the protein curd is produced by heating neutralised whey (pH 6.3-6.5) to 85-100°C followed by acidification to pH 4.0-4.8 (Webb and Hufnagel, 1946). This will precipitate approximately 60-80% of the total protein in whey. The curd can be removed by settling, skimming, filtration (screening) or centrifugation and then dried and packaged.

Many workers have attempted to improve this basic process (Schwartz and Jarczynski, 1940; Peebles and Manning, 1941; Scott and McDonald, 1944; Josh and Hull, 1945, Strezynski, 1945). These works were concerned mainly with the adjustment of heating conditions to increase the yield of the process.

Greater quantities of protein were claimed to be precipitated if calcium chloride was used as the precipitant (Senfton *et al.*, 1950). Rodgers and Palmer (1966) claimed that the best precipitating conditions result by carrying out the heating process in several stages. These include:-

(i) preheating the whey (at pH 4.6) to the stage just before appreciable coagulation takes place;

(ii) heating the whey under conditions of turbulence and in which flocs of precipitated whey proteins are present;

(iii) after 1-10 minutes, the addition of polyelectrolytes which precipitate and stabilise the flocs to a size at which they can be removed by screening or centrifugation.

Saal (1959) designed a semi-continuous system including a self desludging separator for the removal of coagulated protein from whey. A process in which heat denatured protein is produced on a continuous basis has been patented (Pien, 1971). The process includes heating to 65°C in a plate heat exchanger and then to 95°C by direct steam injection; continuous acidification to pH 4.7 and holding for 15 minutes at 95°C; cooling to 35°C in a plate heat exchanger and centrifugal separation of the coagulated proteins in a continuous self desludging separator.

Panzer *et al.* (1976) increased the processing temperature to 120°C and reduced the holding time to 8 minutes. These workers claim that most of the coagulable protein can be removed under these conditions. Modler and Emmons (1975) improved the process yield by heating under very acidic conditions (pH 2.5-3.5) and then adjusting the solution to pH 4.5 prior to precipitation. Presumably, all of the heat

coagulable protein fraction is removed using these conditions. Amantea *et al.* (1974) have prepared soluble whey protein powder by heating under high acid conditions (pH 2.5-3.5) with the addition of ferric chloride. After precipitation, the sediment is washed with dilute hydrochloric acid (pH 2.5), dissolved and dried.

Preconcentration prior to heating the whey is a potential improvement as it would reduce the amount of liquid that has to be heated or removed. The degree to which the whey is concentrated has to be carefully controlled because too high an increase in total solids may reduce the yield of heat coagulated protein (Nielsen *et al.*, 1973). Amantea *et al.* (1974) have shown that preconcentration of whey before heating increased the yield of ferric whey protein.

Industrially, a number of countries have been producing denatured whey protein (traditional lactalbumin) for many years, e.g. it has been produced in New Zealand since 1957 (Mann, 1977). Acid casein whey is heated to 72°C in a plate heat exchanger and then to 95°C by direct steam injection and held for 10 minutes. The precipitated whey protein is then removed using a self-desludging separator. The slurry is washed with cold water and then spray dried (Mann, 1977). The resulting powder has a protein content in excess of 92% and less than 3% lactose. This process, known as the Centri-whey process, can be used to produce a thick slurry of protein which is then added to cheese milk. This system operates in France and increased cheese yields (> 14%) have been reported (Mann, 1971).

2.3.2 Use of Heat-Denatured Whey Protein

The recovery of protein by the traditional method of heat-acid coagulation produces a heat denatured product with medium to high

protein content. By the very nature of the process, the major functional properties of the proteins are lost — the proteins are not able to form foams, they cannot form gels and they are not soluble and therefore, cannot be used in beverages.

Lactalbumin is used in the food industry because of two important characteristics:-

(a) the ability of the proteins to hold water (undenatured whey proteins have no water holding capacity as they are completely soluble);

(b) lactalbumin has an outstanding amino acid profile and, consequently, is an excellent supplement to increase nutritive value.

Traditional lactalbumin is currently being used in baked goods, such as bread and biscuits, and in puffed breakfast cereals (Robinson *et al.*, 1976; Short *et al.*, 1978). Primarily, the lactalbumin is used to fortify the baked goods and breakfast cereals but the ability of the proteins to bind water is also important. The emulsifying capacity and water holding capacity of traditional lactalbumin powder could be put to use in the manufacture of sausages, soups and mayonnaise. Jelen (1974) has investigated the use of traditional lactalbumin as an extender in meat patties and meat loaves.

By taking advantage of the water holding and water binding capacity, traditional lactalbumin could possibly be used to prevent syneresis in a number of products including gravies, yoghurt and cheese.

Schoppet *et al.* (1976) have found that traditional lactalbumin powder is preferred to undenatured whey protein for use in the manufacture of pasta products. Doughs made of soluble whey protein

and durum wheat flour broke down on mixing, became very sticky and plugged the opening of the extruder. No difficulty was experienced in mixing and extruding denatured whey protein — durum wheat doughs. The addition of lactalbumin to pasta to bring the protein content to 20%, significantly increased the nutritive value of the pasta.

Lactalbumin can also be added to yoghurt, processed cheese, chocolate or ice cream (Mann, 1971). The production of a spread using lactalbumin curd or powder has great potential. Ingredients such as bacon bits, chives and cream can be mixed with fresh curd to produce a snack dip (Jelen, 1974).

The spectrum of use of traditional lactalbumin powder could be greatly increased by chemical or enzymatic modification. Jelen and Schmidt (1976) have shown that alkali solubilisation of traditional lactalbumin produces a free flowing, highly soluble powder after dehydration. This powder may find use in the fortification of beverages or any other product that requires a soluble powder for nutrient fortification. At the present time, the use of alkali solubilised traditional lactalbumin as a food ingredient has not been studied because of the possible formation of an unusual amino acid, lysinoalanine, during the solubilisation process. LAL is considered to be potentially toxic and its effect, if any, on the human body has yet to be determined.

2.3.3 Nutritional Value of Whey Protein

Osborne and Mendel (1924) showed that whey protein was a nutritionally superior protein. The availability of amino acids and the limiting amino acids are important factors when considering the nutritional status of a protein. The sulphur containing amino acids (methionine and cystine) and lysine are often the limiting amino acids in most

food proteins.

Undenatured whey proteins and traditional lactalbumin contain a surplus of the majority of the amino acids including the limiting amino acids. Table 4 shows the essential amino acid profiles of a number of whey protein concentrates compared to the suggested FAO pattern. The amino acid profiles compare favorably to the FAO reference pattern. Differences between the profiles could be due to variations in the protein source and/or different processing conditions (Smith, 1976).

Processing conditions can reduce the nutritive value of whey protein. The nutritive value of drum dried whey protein powder was increased by the addition of lysine but no effect was observed with spray dried powder (Riggs *et al.*, 1955). In the presence of reducing sugars, lysine is extremely sensitive to heat damage. The longer drying time and slower removal of moisture during drum drying of lactalbumin could contribute significantly to the loss of lysine.

The availability of lysine in traditional lactalbumin will vary with the drying method. Centrifugally separated and spray dried lactalbumin contained 94-96% available lysine whereas lactalbumin produced by decantation and drying in a casein dryer contained 82% available lysine (Robinson *et al.*, 1976).

Traditional lactalbumin is a nutritionally superior protein (table 4), has a high protein efficiency ratio (2.92 as compared to 2.5 for casein) and, therefore could be of value in supplementing proteins of lower nutritional value (e.g. cereal grains). The addition of 4% traditional lactalbumin to white flour increased the protein efficiency ratio (PER) of the flour from 0.83 to 2.05 (Robinson *et al.*, 1976).

Table 4: Essential Amino Acid Profiles (g/100 g Protein) of Several Whey Protein Concentrates

Amino Acid	FAO[a] Pattern	Ultra-[a] Filtered	Gel-[a] Filtered	Chemically[a] Precipitated	Lactal-[b] bumin
Isoleucine	4.2	9.9	9.9	5.5	5.06
Leucine	4.8	6.2	-	11.5	11.96
Lysine	4.2	8.6	9.7	9.2	9.23
Phenyla-lanine	2.8	3.1	3.1	3.8	3.53
Tyrosine	2.8	2.9	2.9	3.5	3.79
Cystine	2.0	6.0	2.4	2.5	3.87
Methionine	2.2	2.4	1.9	1.8	2.46
Threonine	2.8	5.7	5.7	5.6	5.14
Tryptophan	1.4	-	-	-	2.38
Valine	4.2	6.7	6.5	5.5	5.75

(a) Smith (1976)

(b) Robinson *et al.* (1976)

The addition of traditional lactalbumin to corn and rice protein almost doubled the PER. Even after moderate heat treatment, the lactalbumin-cereal mixtures still had a high protein quality (Forsum, 1974; Robinson *et al.*, 1976).

2.4 Water Relations of Whey Protein Concentrates

Regardless of which method is used to produce denatured whey protein curd, there is an irreversible loss in WHC upon drying. The role of water in foods, in particular the hydration of whey proteins, will now be discussed.

2.4.1 Water in Foods

The structure of pure water has been reviewed by a number of authors (Nemethy, 1968; Frank, 1970; Tait and Franks, 1971; Stillinger, 1977) and, as Labuza (1977) has shown, there is still considerable conflict about the structure of liquid water. Certain model structures — the continuum model theory as an example — state that water exists as a heterogeneous mixture of monomeric and polymeric structures (Frank, 1970). Other models conclude that liquid water exists as an ice like structure (Franks, 1975) and Nemethy (1968) suggests that liquid water is a mixture of clusters of monomeric and polymeric units. All models explain some of the properties of liquid water, but it wasn't until 1977 that Stillinger showed that all liquid water properties could be explained using the continuum model.

In foods, water can exist in a number of forms. These include free (surface) water, water of hydration, water that is chemically bound to salts and water that is adsorbed as mono- or polymolecular layers. The latter two forms of water in food are commonly called "bound" water — a term that has been misused and misunderstood since

Gortner and his co-workers introduced the concept in the 1930's. A study of the literature has shown that there is no acceptable definition for "bound" water although Kuprianoff (1958) suggests that bound water can be considered as *...that part of the water content of a product which remains in it in an unchanged state after the application of unusual drying procedures...which can only be expelled by heating to 100-110°C for a sufficiently long time...*

Twenty years later, most of the definitions still say essentially the same thing. Kuntz and Kauzman (1974) state that any water whose properties differ detectably from those of the "bulk" water in the system, could be considered bound.

Water is by far the dominant and most important component of foods. The role of water in food depends on the chemical and physical composition of the food (Kuprianoff, 1958). Water influences the physical, chemical, mechanical and enzymatic behavior of foods. Water is able to influence food texture e.g. fruits lose their crispness when water is removed and crackers become soft when water is absorbed.

When microbiological problems are eliminated, the storage life of foods becomes limited due to chemical reactions. The concept of bound water now becomes of special importance when the bulk of the water is removed by freezing, dehydration or chemical binding. By definition, bound water, for the purposes of this work, is considered to be the monolayer of water that is bound to the active sites of the substrate. This monolayer water is considered to be tightly bound and does not behave as "free" water.

Above the monolayer region, water may be able to act as a solvent, reactant and diluent. As the amount of water sorbed increases, hydrolytic

reactions such as enzyme hydrolysis increase. Water can also act as a "protectant" in foods; below the monolayer region, oxidative reactions increase at a dramatic rate because water is not able to provide a "protective covering" at the active sites.

The application of an operational definition for "bound" water is difficult — the determination of "bound" water is even more difficult. The state of water in foods has been studied by a number of different techniques — e.g. unfreezable water determination by differential scanning calorimetry (Berlin *et al.*, 1973); thermogravimetry (Berlin *et al.*, 1971); nuclear magnetic resonance (Shanbhag *et al.*, 1970; Leung *et al.*, 1976); infra red and Raman spectroscopy (Franks, 1975); unfreezable water determination by differential thermal analysis (Duckworth, 1975; Simatos *et al.*, 1975); dielectric analysis (Kent and Jason, 1975) and water vapor sorption techniques. Excellent reviews on these techniques have been published by Ling (1972), Kuntz and Kauzman (1974), Duckworth (1975) and Chirife and Iglesias (1978).

2.4.2 Sorption Characteristics

One of the easiest methods available for the determination of some of the properties of water in a food, is the measurement of equilibrium relative humidity. An important concept to understand is the water activity of a system which is commonly expressed as:-

$$a_w = p/p_o$$

where a_w = water activity

p = vapor pressure of food

p_o = vapor pressure of water at the same temperature

A plot of water content against the water activity is referred to as a moisture sorption isotherm. An analysis of the isotherm will

yield information regarding the binding of the water in foods.

A number of theoretical approaches for the binding of water onto a solid substrate have been developed. In general, they involve the adsorption of water vapor onto internal or external surface "binding" sites or the condensation of water in capillaries (Kuprianoff, 1958; Kuntz and Kauzman, 1974). Chirife and Iglesias (1978) have prepared an excellent review on the equations available for the analysis of isotherms. A number of important points are emphasised:-

(i) most adsorption theories ignore changes in the substrate surface (very important with water-protein systems);

(ii) there is no mathematical model which will describe the whole isotherm;

(iii) information from the isotherm must be interpreted within the limitations imposed by the sorption theory used.

In 1938, Brunauer *et al.* developed their multimolecular adsorption theory and, from this, a classification scheme for isotherms was formulated. There are five types of isotherms. As a generalisation, type II and type III isotherms apply to a non-porous solid material with an infinitely thick adsorbed layer. To be more specific, sugars will resemble a type II or III isotherm because they go into solution — not because they fix an infinitely thick layer of water. Usually the type II isotherm approaches infinity when a_w approaches 1. Type IV and type V isotherms are associated with porous materials where the thickness of the internally adsorbed layer is limited by the pore diameter (Gregg and Sing, 1967).

Most foods, and all high protein foods, resemble the type II isotherm (Labuza, 1968; Kuntz and Kauzman, 1974).

An idealised isotherm can be divided into three regions: a region of monomolecular adsorption, a region of moisture sorption on top of the monolayer region, and a region corresponding to capillary condensation. These regions are not homogeneous and there can be considerable overlap (Labuza, 1968). A hysteresis effect, common with the isotherms of many foods, is observed when the desorption isotherm is higher than the adsorption isotherm. The effect usually ends at the monolayer (Labuza, 1968) although it can extend down to a water activity of zero. Several theories have been proposed to explain this effect and none are entirely satisfactory. Labuza (1968, 1977) suggested that the difference in contact angle (between adsorption and desorption) of water sorbed in the pores can explain this effect.

As stated earlier, many quantitative theories have been developed to explain sigmoidal isotherms. The theory developed by Brunauer, Emmett and Teller (1938) — known as the BET theory — was the first to be widely accepted in most areas of research. The BET theory postulates a layer of independent substrate water binding sites similar to those described by Langmuir (1918). Additional adsorption layers are permitted which bind more weakly than the first layer. The assumptions behind the BET theory are:-

(i) the energy of sorption for all molecules in the first layer is the same and is equal to the heat of vaporisation plus a constant heat due to site interaction;

(ii) the energy of sorption in all subsequent layers is the heat of vaporisation;

(iii) more than one layer of sorbate molecules may be present on the surface;

(iv) adsorption occurs only at specific sites;

(v) when the vapor pressure of the system reaches saturation vapor pressure, the sorbate molecules will condense on the liquid film.

The application of the BET theory to sigmoidal isotherms enables the calculation of the following quantities:-

(a) BET monolayer value: this is the quantity of water bound to the active sites of the substrate;

(b) BET surface area: the surface area that the monolayer water covers;

(c) BET heat of sorption: this quantity represents the heat of site interaction; the difference between heat of adsorption and heat of desorption (if any) could possibly be attributed to the heat of swelling of the substrate.

The equations used to calculate the above three quantities are given in sections 5.2.2, 5.2.3 and 5.2.4.

2.4.3 Protein Hydration

Pauling (1945) accepted the basic assumption of the BET theory (namely, that water taken up by the proteins beyond the first layer exists as normal liquid water) and came to the conclusion that each polar group binds one molecule of water. In other words, the number of polar groups is similar to the number of water molecules initially adsorbed.

When considering the hydration of solid proteins, the following reaction sites are thought to be involved (Ling, 1972; Kuntz and Kauzman, 1974):-

(i) the hydroxyl (on serine, threonine and tyrosine side chains), the carboxyl (aspartic, glutamic and α-carboxyl), and the basic groups (arginine, histidine, lysine and α-amino groups) all bind water except when they are in the presence of an amide side chain which is thought to form a hydrogen bond with the non-amide side chain polar group and, in doing so, eliminates the water binding capacity of the latter;

(ii) there is strong evidence to suggest that the peptide imino and carbonyl groups are inherently capable of binding large quantities of water, however, these sites are generally ignored as water binding sites because of other factors such as intra- or intermolecular hydrogen bonding of the protein chains, which may prevent the occurrence of water binding.

These points represent the backbone of the theory of Bull and Breese (Ling, 1972) and, fundamentally, they are very similar to Pauling's assumptions. The theory of Bull and Breese differs from Pauling's in two ways (Ling, 1972; Kuntz and Kauzman, 1974):-

(a) water sorption is considered at a relative humidity of 92% instead of that corresponding to the initial first layer of water sorption (as calculated by the BET theory);

(b) six water molecules (instead of one) are considered to be "bound" to each polar side chain.

The amount of water bound per gram of protein, as calculated by the theory of Bull and Breese, is in excellent agreement with known experimental hydration capacities of a number of proteins (Ling, 1972). The hydration capacities of bovine serum albumin and β-lactoglobulin

(as calculated by this theory) are 0.28 and 0.29 g water/g dry protein respectively.

The interaction between water and non-polar side chain groups is a subject of disagreement. Most proteins contain large quantities of amino acids with non-polar side chains. These include benzyl (phenylalanine), isopropyl (valine) and secondary butyl and iso-butyl groups (leucine). The interaction between water and these groups is known to have an important bearing on the structure or native conformation of many proteins (Kuntz and Kauzman, 1974).

When a protein is brought into contact with water, the hydrophobic non-polar side chains tend to associate in order to minimise their contact with the water — an effect known as hydrophobic bonding (Fennema, 1975). It is generally thought that water becomes structured when hydrophobic bonds remain in contact with the water and it is thought that this structuring of water decreases the stability of the protein (Lumry, 1973; Kuntz and Kauzman, 1974; Fennema, 1975). The effect of hydrophobic bonding on the structure of water surrounding the protein has been well documented but the effect, if any, on the water binding capacity of a protein has not been established.

Water molecules have been shown to occupy "clefts" and "holes" in the protein structure (Lumry, 1973). These are bound by strong hydrogen bonds and are considered to be an integral part of the protein. It could be hypothesised then that dislocation of water which occurs during dehydration would offer an opportunity for additional inter- and intramolecular protein bonding, including the hydrophobic bond.

The strength of the hydrophobic bond decreases as the temperature

is lowered (Fennema, 1975). Assuming the preceding hypothesis to be true, it could be further hypothesised that the degree of intra- and intermolecular hydrophobic bonding would vary according to the drying method employed. In other words, protein products dried by a method such as drum drying which employs severe heat treatment, could be expected to have a larger degree of inter- and intramolecular hydrophobic bonding than products dried by a less severe method (freeze drying). It is possible, then, that the water binding capacity (WBC) of a drum dried protein product could be lower than the WBC of the same product if it were freeze dried.

2.4.4 Water Sorption in Whey Protein Concentrates

Water binding in undenatured whey protein products has been extensively studied by Berlin *et al.* (1968a, 1968b, 1970, 1971,1973). The results obtained by these workers are summarised below:-

(i) in whole whey powder, water is primarily bound by the protein fraction at a low water activity; as the water activity increases to above 0.50, lactose becomes more active in the water vapor sorption;

(ii) above a water activity of 0.50, the salts present in whey powder and undenatured whey protein concentrate become actively involved in water sorption;

(iii) analysis of water binding in whey powder, β-lactoglobulin and lactose glass shows that water is sorbed through primarily weak binding forces at low to intermediate relative humidities;

(iv) water vapor sorption in whey powders and undenatured whey protein concentrates obeys the law of additivity; it is a

function of the amount of water sorbed by protein, lactose, ash and other minor components present.

Bound water in spray dried, undenatured protein solutions containing lactose and salts varied from 0.50 to 1.20 g water/g solids (Berlin *et al.*, 1973). Water vapor sorption was found to be an additive process at all relative humidities with the amount of bound water increasing as the concentrations of lactose and salts increased.

Because a universal definition for "bound water" does not exist, values obtained for "bound water" determinations will vary considerably with the method chosen to measure this quantity. The BET method used in this work will, invariably, give considerably lower results than "bound water" as determined by unfreezable water content. Because of this, the BET method may not be a true measure of the "bound water" because physical and chemical attraction of water to the substrate could occur over and above the monolayer. This is especially true with proteins. The fundamental assumption of the BET method is that "bound water" is considered to be a monomolecular layer and that one molecule of water will bind to one active site. With proteins, the BET monolayer region varies from an a_w of 0.15 to 0.41 but Bull and Breese (Ling, 1972) have shown that most proteins are not fully hydrated until an a_w of 0.92 and that six molecules of water are bound to each active site.

When proteins absorb water, they swell and will eventually form a thermodynamically stable gel-like structure. The degree of protein swelling can be related to water vapor sorption in whey protein concentrates. It was found that sorption at low and intermediate water activities increased with the ability of the proteins to swell and take

up water (Hermansson, 1977). The degree of swelling was shown to be pH dependent with a maximum occurring at pH 6-8 (Hermansson; 1972, 1973).

So far, the studies discussed have been confined mostly to undenatured whey protein concentrates or whey powders. Very few studies have been concerned with the water holding capacity of traditional lactalbumin and none, to the author's knowledge, with the water sorption properties of traditional lactalbumin.

Knightbridge and Goldman (1975) measured the water absorptive capacities of various dried milk products using the Brabender farinograph. Because traditional lactalbumin powders contain soluble constituents which have no effect on the water absorptive capacity once solubilised, the result is often reported on a protein basis only. Traditional lactalbumin was found to have a water absorptive capacity of only 96 g water/100 g dry protein and, on this basis, was classified as a low absorption product. Using this method, the water absorptive capacities of sodium caseinate and calcium caseinate were 295 and 159 g water/100 g dry protein respectively. Skim milk powder ranged from 96-128g water/100 g dry protein depending on the heat treatment received prior to drying.

Short *et al.* (1978) showed that traditional lactalbumin made from different types of whey had similar water absorption capacities. Spray dried traditional lactalbumin had the highest absorption capacity (approximately 180 g water/100 g dry protein) whereas drum dried traditional lactalbumin was substantially lower (100 g water/100 g dry protein). Changes in WHC as affected by the drying method altered the texture and color of biscuits incorporating traditional lactalbumin.

In summary, this review has examined the current knowledge on whey protein as it progresses from the raw material to the processed product. Whey protein concentrates can be produced by a number of different methods but one of the simplest, and least expensive, is the traditional method of heat-acid coagulation. The product produced by this method is a bland, white, insoluble, high protein curd which can be used as is, or dried and used as a powder. The product is nutritionally superior to many protein sources and finds use as an additive in many areas of the food industry.

Prior to dehydration, the denatured protein curd has a high water holding capacity. It can contain as much as 70-75% water and still appear dry and crumbly. But, when the curd is dried, there is an irreversible loss in the WHC. This loss in WHC has been known for many years (Josh and Hull, 1945; Gillies, 1974) but nobody, to the author's knowledge, has investigated why this loss occurs. For this reason, the water holding and water sorption studies discussed in this work were initiated in an attempt to elucidate this problem.

CHAPTER 3

PREPARATION AND PROXIMATE ANALYSIS OF EXPERIMENTAL MATERIALS

The heat-acid coagulation method was used at the Department of Food Science, University of Alberta, to produce several batches of whey protein curd. Dehydration of the curds obtained by the heat-acid coagulation process was accomplished by using five different methods: freeze drying, spray drying, drum drying, air drying and vacuum drying. These methods were chosen in an attempt to determine the effect of drying method on the WHC and WBC of the powders. Each powder produced was analysed for residual moisture, protein, lactose, ash, pH and bulk density.

3.1 Experimental Materials

3.1.1 Preparation of Whey Protein Curd

Cottage cheese whey was collected from the Lucerne Dairy Company, Edmonton. A sample of each shipment was taken and pH and total solids were measured. A 200 litre batch of whey was neutralised to pH 6.2-6.5 using approximately 300 ml of 40% sodium hydroxide and then heated in a jacketed vessel. Temperature inside the jacket of the heating vessel was maintained at 150°C during the heating and holding stages. When the whey reached 95°C, the pH of the solution was reduced to pH 4.2-4.8 (Webb and Hufnagel, 1946) by the addition of approximately 300 ml of glacial acetic acid. The temperature was then maintained at 95°C for 20 minutes. Continuous stirring was in progress during the neutralisation, heating, acidification and holding stages.

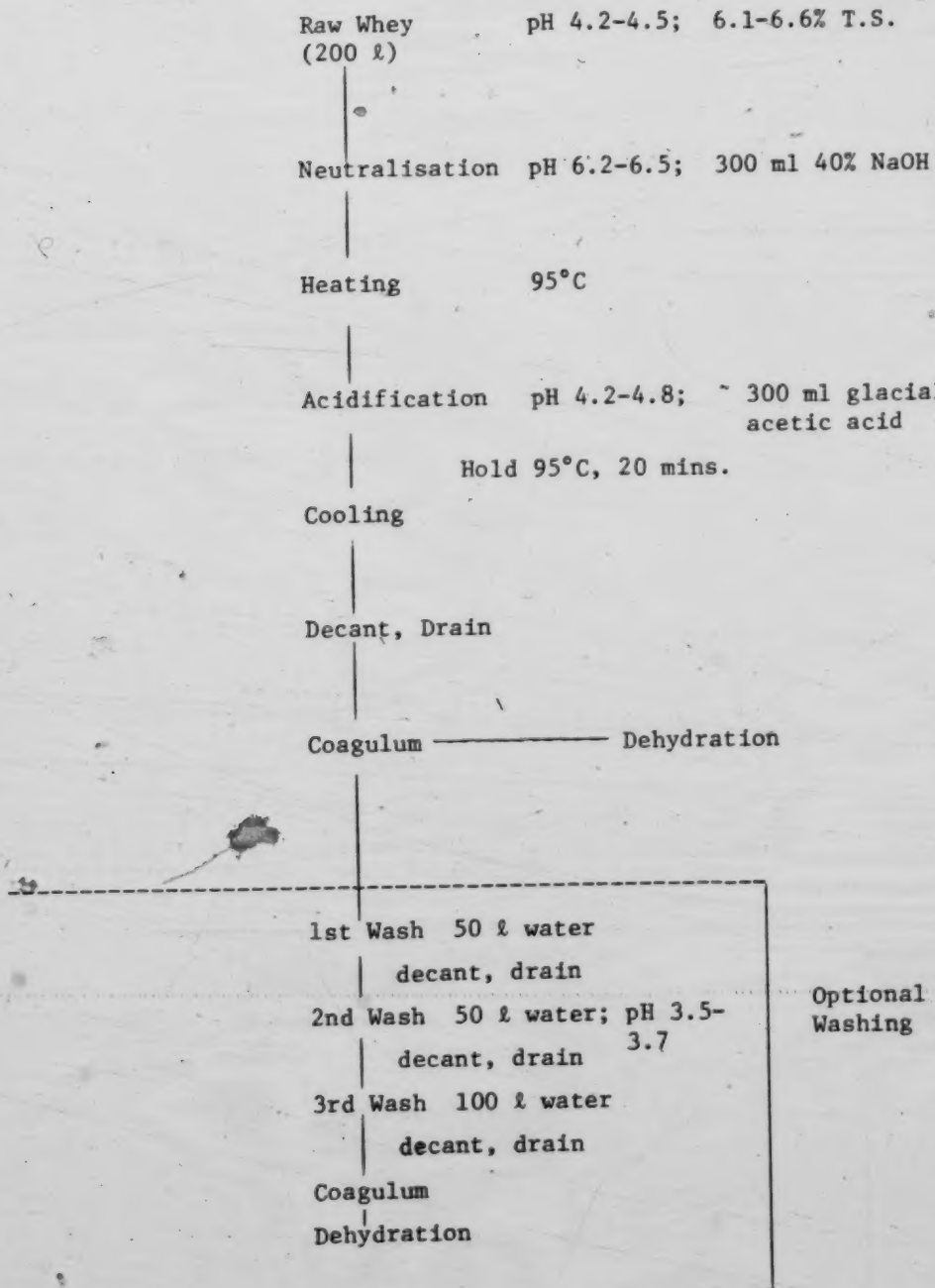
After the holding period, the product temperature was slowly reduced to approximately 10°C and stirring was stopped. When the coagulum

had settled, the top layer was decanted and the coagulum was drained into several cotton bags. After approximately 4 hours drainage, the dry crumbly curd (approximately 80% moisture depending on the humidity in the room on the day the curd was drained) was dried by one of the five chosen methods; either directly or after washing.

If the curd was to be washed, it was returned to the jacketed vessel and washed with 50 litres of cold (10-15°C) water. The curd was stirred for 20 minutes, decanted and drained as above. A second washing step involved the addition of 50 litres of cold (10-15°C) water followed by acidification to pH 3.5-3.7 using 2 N hydrochloric acid. This second washing stage was used to reduce the ash content of the curd. The calcium-phosphate complex is soluble at this pH and the ash can typically be reduced from approximately 15% (unwashed) to 2-3% (Harwalkar and Emmons, 1969). A third washing step, introduced after the acid wash, restored the pH of the curd to 6.0-6.5. About 100 litres of water were added to the drained coagulum from the second wash and the product was stirred for 10 minutes, decanted and drained. Figure 2 is a schematic representation of the heat-acid coagulation process used.

By deleting the washing step entirely or by using a combination of one or more washing stages, it was possible to produce powders with a range of protein, lactose and ash contents. These powders were then used for subsequent determination of water holding capacities and sorption characteristics. Eight batches of denatured protein curd were produced. The first three batches were used to establish and familiarise the author with the conditions of the process. The last five batches were used for subsequent WHC and WBC studies.

Figure 2: Heat-Acid Coagulation Process



3.1.2. Preparation of Reference Materials

Three reference materials (dialysed denatured whey protein, dialysed undenatured whey protein and lactose glass) were prepared for the water sorption study (Chapter 5).

(a) Dialysed Undenatured Whey Protein

Five litres of cottage cheese whey were extensively dialysed against deionised, distilled water to remove most of the lactose, mineral ions and salts. The temperature was maintained at 5°C for the duration of the dialysis procedure. After 7 days, the protein solution was freeze dried using a Virtis Company freeze dryer (The Virtis Co., N.Y., Model FFD-42-WS). The dried protein powder was then analysed for protein, lactose and ash.

(b) Dialysed Denatured Whey Protein

A batch of denatured whey protein curd was prepared and washed using the procedures outlined in section 3.1.1. The washed curd was suspended in deionised, distilled water and dialysed as for the undenatured protein. After dialysis, the curd was freeze dried and analysed for protein, lactose, ash.

(c) Lactose glass

A 15% w/w solution of lactose (Fisher Scientific, Analytical Grade Reagent) in water was prepared and drum dried using a Blaw-Knox Co. double drum dryer (Blaw Knox Co., Buffalo, N.Y., Model No. AL1-4). After the sheet of dry lactose had cooled, it was ground using a mortar and pestle and stored in a 0% relative humidity dessicator.

3.2 Dehydration of Whey Protein Curd

Five different drying methods were used to determine their effect (if any) on the WHC of the denatured whey protein powder.

(a) Freeze Drying. Samples of each curd were freeze dried in a Virtis Company freeze dryer (The Virtis Co., N.Y., Model FFD-42-WS). The product was thinly spread (4-6 mm bed depth) on a stainless steel drying tray and frozen to -50°C. A vacuum of 5-10 μ, plate temperature of 25°C and drying time of 24 hours was used.

(b) Drum Drying. The moisture content of the coagulum was determined using the vacuum oven method (section 3.3.1). Water was then added to the coagulum to produce 2000 g of a 10% solids slurry of the coagulum. The slurry was blended in a Waring Blender for 30 seconds to remove any large lumps of curd. The resulting suspension was then dried using a Blaw-Knox Co. double drum dryer (Blaw-Knox Co., Buffalo N.Y., Model No. AL1-4). The following operating conditions were employed:

(i) drum speed; 1.5 rpm

(ii) drum steam pressure; 70 psig

(iii) slurry flow rate; 125 ml/minute.

(c) Spray Drying. A 10% solids slurry was prepared as for drum drying. The spray dryer was a conical type laboratory spray dryer (Bowen Engineering Inc., North Branch, New Jersey) and was operated under the following conditions:

(i) inlet air temperature; 285°C

(ii) outlet air temperature; 95°C

(iii) product flow rate; 65 ml/minute

(iii) product flow rate; 65 ml/minute

(iv) air pressure at atomiser nozzle; 80 psig.

(d) Air Drying. Two air drying methods were used. Air drying at 25°C was carried out in a Constant Temperature Cabinet (Labline Inc., Chicago Ill.). Sorbic acid (0.05% w/w) was added to the product to restrict the growth of bacteria and mould. The coagulum was spread thinly (bed depth approximately 1-2 mm) on an aluminum tray and left to dry for 48 hours.

Air drying at 40°C was achieved by using a forced air convection oven (Fisher Isotemp Oven, Fisher Scientific Co. Ltd., Edmonton, AB.). The coagulum was thinly spread on an aluminum tray (1-2 mm bed depth) and dried for 36 hours.

(e) Vacuum Drying. The coagulum was thinly spread (1-2 mm bed depth) on an aluminum tray and dried using a vacuum oven (National Appliance Co.). The coagulum was dried at 60°C and 29" Hg vacuum for 36 hours.

All powders were ground with a mortar and pestle and size graded using a Canadian Standard Sieve series (W.S. Tyler Co., St. Catherines, Ont.). The 60# size fraction ($<250\mu M$ but $>180\mu M$) was used in the water holding and water sorption studies. Table 5 summarises the residual moisture contents of each powder.

Table 5: Residual Moisture Contents of Denatured Whey Protein Powders

Drying Method	Moisture Content % w/w
Freeze Drying	1.5 - 4.5
Drum Drying	8.0 - 9.5
Spray Drying	3.0 - 5.0
Air Drying (25°C)	8.0 - 10.0
Air Drying (40°C)	6.0 - 8.0
Vacuum Drying	< 1.5

3.3 Proximate Analysis of Powders

Powders produced from each batch of coagulum were analysed for moisture, protein, lactose, ash, pH and bulk density.

3.3.1 Methodology

(a) Moisture Content

The moisture content of each powder was determined using the vacuum oven method. Samples were dried at 70°C under 29" Hg vacuum for 48 hours or until constant weight was reached. Each determination was performed in triplicate.

(b) Lactose

Lactose was determined using the anthrone method developed by Fagen *et al.* (1954). Dilute suspensions of each powder were prepared and treated with the anthrone reagent. After heating, color development was measured at 580 nm using a Bausch and Lomb Spectronic 20. The lactose content of the powders was determined using a standard curve prepared from solutions of known lactose content (lactose supplied by Fisher Scientific, Analytical Grade Reagent).

(c) Protein

Total nitrogen was determined using the semi-micro kjeldahl method (Pearson, 1970). Protein was calculated by multiplying the nitrogen percentage by a factor, 6.38, which is customary in protein determinations of all dairy products (A.O.A.C., 1975).

(d) Ash

Ash was determined using the method outlined in A.O.A.C. (1975). Approximately 5 g of sample was first dried under

vacuum (70°C, 24 hours, 29" Hg) and then ashed at 550°C to a constant weight (approximately 2-3 hours).

(e) pH

A 6% (w/w) suspension of each powder was prepared using distilled water and the pH (25°C) was measured using a Fisher Acumet (Model 320) pH meter.

(f) Bulk Density

The bulk density of all powders was determined using the method of Ooraikul (1973). The powder was poured into a 100 ml measuring cylinder to the 100 ml mark and tapped (NOT PACKED) until no further settling of the powder occurred. After weighing the powder occupying the 100 ml volume, the bulk density was calculated.

3.3.2 Results

Results for protein, lactose, ash, water and pH determinations of the five experimental batches are given in table 6. The effect of washing on the water soluble lactose and pH can be seen in this table. Powders 1 and 2 were unwashed and contained 25-30% lactose; powder 3 was washed once (16% lactose) and powders 4 and 5 were washed three times (5-6% lactose). When all three washing stages were used, protein contents increased to approximately 90%. Typically, industrially prepared denatured whey protein contains 80-90% protein, 1-4% ash, 1-7% lactose and up to 6% lipid material (Harwalkar and Emmons, 1969; Robinson *et al.*, 1976). As expected, unwashed powders (1 and 2) had a more acidic pH than washed powders.

The effect of drying method on bulk density can be seen in table 7. Freeze dried powders had lower bulk densities than drum dried, air dried,

Table 6: Proximate Analyses of Freeze Dried Denatured Whey Protein Powders

Batch No.	Protein[a] (%)	Lactose[a] (%)	Ash[a] (%)	Water (%)	pH (25°C)
1 (unw)[b]	64.81	29.41	4.46	2.75	4.74
2 (unw)[b]	69.06	25.03	5.12	1.24	4.84
3 (w)[b]	78.01	16.65	3.43	1.36	6.51
4 (w)[c]	91.68	5.83	2.15	2.84	6.58
5 (w)[c]	90.37	6.79	3.08	1.93	6.49
Dialysed denatured whey protein	98.15	d	0.85	-	-
Dialysed undenatured whey protein	98.37	d	1.06	-	-

Note: (a) Dry weight basis

(b) Unwashed whey protein powder

(c) Washed whey protein powder

(d) Lactose not detected

Table 7: Bulk Densities of Dried Whey Protein Powders

Batch No.	Bulk Density (g/cm^3)
1	0.32
2	0.33
3	0.41
4	0.36
5	0.39
5; spray dried	0.56
5; drum dried	0.55
5; air dried (25°C)	0.80
5; air dried (40°C)	0.79
5; vacuum dried	0.68

Note: All of the above samples are freeze dried except those indicated as being dried by a different method.

vacuum dried and spray dried powders. Considering the fact that all of the freeze dried powders (1 to 5) were produced under identical conditions, the possible effect of product composition on bulk density cannot be neglected. The low bulk density powders (1 and 2) also had the lowest protein contents (65-69%) and the highest lactose contents (25-30%).

CHAPTER 4

WATER HOLDING STUDY

4.1 Introduction

Chapters 4 and 5 are concerned with the adsorption and absorption properties of the various powders. This chapter examines the dehydration method, product composition and physical differences in the powders and how each of these variables affects the water holding capacity of the powder in question. Chapter 5 deals with the water sorption studies carried out on the different powders. The water sorption study was undertaken to see if differences in sorption characteristics (BET monolayer value, BET surface area, BET heat of sorption) could be related to differences in WHC. The two chapters are interrelated and should not be considered as separate units.

4.2 Methodology: The Water Holding Capacity Test

Fourteen different powders, produced as outlined in section 3.2, were used for both the water holding study and the water sorption study so that comparisons could be made.

The powders to be tested for WHC were vacuum dried (48 hours, 60°C) to remove any residual water. The WHC of the powders was determined using a method developed by Medcalf and Giles (1965) and Morrow and Lorenz (1974) for use in WHC studies on starch, modified as follows:

After dehydration, 2.5 g of dry powder and 37.5 g of water were accurately weighed into a 50 cm^3 centrifuge tube. The tubes were inverted two or three times to disperse any large lumps. After inversion, the tubes were clamped into a "wrist action" shaker (Burrell Corp., Pittsburg, Pa.) and mixed for 60 minutes at the rate of 1 cycle/second.

When the mixing was completed, the tubes were removed and centrifuged for 20 minutes at 900 x g using the International Centrifuge (International Equipment Co., Boston, Mass.). The supernatant was then decanted, the tubes were inverted and drained for 10 minutes and weighed. The WHC was expressed as weight of water retained per 100 g dry protein. Each WHC test was determined in quadruplicate and tests were conducted at 5°C and 25°C to see if test temperature would affect the WHC of a powder.

The initial WHC of the wet curd (prior to dehydration) was determined using the same method. The solids content of the curd was calculated using the vacuum oven method and sufficient curd was added to a centrifuge tube so that 2.5 g of solids were present. Water was then added to the tube so that the solids-water ratio in the wet curd was the same as in the dry powder tests. The tubes were then mixed, centrifuged, decanted and weighed as described above.

4.3 Results and Discussion

The method used to measure the WHC of denatured whey protein is reasonably quick, accurate and very reproducible. Reference to tables 8, 9 and 10 shows that, in the majority of analyses, the standard error is less than 2%. One potential problem is that it may give a marginally higher value for the WHC of the powders when compared to other methods.

Using the farinograph method developed by Knightbridge and Goldman (1975), Short *et al.* (1978) reported water absorption values in the range 175-180 g water/100 g dry protein for spray dried traditional lactalbumin curd. Values in the range 240-260 g water/100 g dry protein (table 9) were obtained for spray dried curd in this work.

It is not known whether the differences between the two methods

are due to inherent differences in the methods, differences in the powders or due to a combination of both effects. It is most likely that the largest single contributing factor to WHC differences (as determined by both methods) is the difference in the porosity and particle size distribution of the spray dried powders.

Even though the method used here gave marginally higher WHC values, as a comparative method it was useful for determining differences in WHC of various protein powders. It should be stressed again that Knightbridge and Goldman (1975) classified lactalbumin as a low absorption product. In this work, traditional lactalbumin powders had water holding capacities that ranged from 200-750 g water/100 g dry protein depending on the drying method.

4.3.1 Effect of Product Composition on WHC

Table 8 summarises the effect of product composition on the WHC of denatured whey protein powders. Table 6 is a summary of the proximate analyses of the powders and should be used in conjunction with table 8. Three important trends can be noted in table 8:

(a) the test temperature appears to affect the WHC: the lower the temperature the higher the WHC;

(b) low protein powders (less than 70% protein) appear to have a higher WHC than high protein powders;

(c) powders with a more acidic pH seem to have a higher WHC than powders with a pH approaching neutrality.

Data in table 8 were statistically analysed on computer by two-way analysis of variance (ANOVA) using a standard program supplied by the University of Alberta.

At the 1% level of significance, there was a significant effect of

Table 8: Effect of Composition on WHC of Denatured Whey Protein Powders

Batch No.	WHC (5°C) g water/100 g dry protein	WHC (25°C) g water/100 g dry protein
1	$^{a}739 \pm 17.0$	$^{d}659 \pm 11.0$
2	$^{a}754 \pm 3.0$	$^{d}676 \pm 4.5$
3	$^{b}558 \pm 8.0$	$^{e}539 \pm 9.5$
4	$^{c}588 \pm 10.0$	$^{f}577 \pm 6.0$
5	$^{b}560 \pm 8.5$	$^{e}545 \pm 9.0$

Notes: (i) Batch 1, 2 unwashed; Batch 3 washed once; Batch 4, 5 full wash.

(ii) Values in the same column preceded by the same superscript are not significantly different ($P \leq 0.05$). The differences (a,d) and (c,f) were significant at the 1% and 5% levels respectively.

(iii) All powders were freeze dried.

test temperature on WHC. The lower WHC at higher temperature could be due to a number of factors including:

(i) decrease in protein swelling at higher temperatures (Hermansson, 1972; Iglesias and Chirife, 1976a);

(ii) expansion of the water molecules due to an increase in temperature (Brunauer *et al.*, 1938);

(iii) a reduction in the number of active sites available for water adsorption as a result of physical and/or chemical changes induced by temperature.

The data in table 8 show a possible effect of pH on the WHC of the powders. The apparent increase in WHC with lowering of pH may be purely coincidental when considering the effect of pH on protein hydration. When a protein is denatured, there is an unfolding of the polypeptide chain with the subsequent exposure of hydrophilic groups that were buried within the tertiary structure framework. This exposure of buried hydrophilic groups will only slightly increase the water binding capacity of globular proteins. When the pH is reduced, the protonation of the exposed carboxyl groups will prevent them from combining electrostatically with water. Thus, as the pH is reduced, the water binding capacity of the protein in question should decrease (Tanford, 1961; Kuntz and Kauzmann, 1974). Evidence for this reduction can be seen in the monolayer values of denatured whey protein powders that had different pH values (section 5.3.3). As an example, freeze dried batch 2 (pH 4.84) had a monolayer value of 4.73 g water/100 g dry solids whereas freeze dried batch 3 (pH 6.51) had a monolayer value of 8.73 g water/100 g dry solids. Furthermore, Hermansson (1973) has shown that the swelling ability of whey protein concentrates is higher at pH 6 than

at pH 4.

In light of this, it would be expected that there would be a decrease in WHC with decrease in pH. Results show that this is not so and this leads to the speculative conclusion that the pH differences were purely coincidental. It should be noted that the pH of powders 1 and 2 would be naturally lower than in powders 3, 4 and 5. Powders 1 and 2 were not washed and still contained most of the salts, lactose, etc.

There was a significant difference in WHC between batches (table 8). Batches 1 and 2 had significantly higher water holding capacities ($p \leq 0.01$) than batches 3, 4 or 5 at both test temperatures. There are two possible reasons for this difference:

(a) All water holding capacities were calculated on a dry protein basis assuming that the lactose would dissolve leaving only the denatured protein in the pellet after centrifugation. It is quite possible that, in the high lactose powders (1 and 2), a substantial amount of lactose was left in the pellet. This lactose could contribute to the dry weight of the pellet and the results would be erroneously high.

(b) Powders 1 and 2 had lower bulk densities than powders 3, 4 or 5.

The WHC differences observed in table 8 are not an effect of drying (because all were freeze dried under the same conditions) but could be attributed to differences in powder composition and/or bulk density.

4.3.2. Effect of Drying Method on WHC

Data in table 9 were statistically analysed by two-way analysis of variance which showed a significant effect of drying method and batch

Table 9: Effect of Drying Method on WHC of Denatured Whey Protein Powders (25°C)

Drying Method	WHC (g water/100 g dry protein)		Average % retention of WHC
	Batch 4	Batch 5	
Freeze Dried	$^{c}577 \pm 6.0$	$^{d}545 \pm 9.0$	78
Drum Dried	$^{b}253 \pm 7.0$	$^{e}254 \pm 11.5$	33
Spray Dried	$^{b}249 \pm 6.0$	$^{e}251 \pm 1.0$	31
Air Dried (25°C)	$^{a}195 \pm 2.0$	$^{f}182 \pm 6.0$	26
Air Dried (40°C)	$^{b}263 \pm 3.0$	$^{e}240 \pm 1.0$	40
Vacuum Dried (60°C)	$^{b}257 \pm 8.0$	$^{e}249 \pm 4.0$	34

Notes: (i) Initial WHC of the fresh curds was 756 ± 2.5g water/100 g dry protein.

(ii) Values followed in the same column by the same superscript are not significantly different ($P \leq 0.05$). The differences (a,c,d,f) were significant at the 1% level.

on WHC of denatured whey protein powders. Each batch (4 and 5) was then analysed individually by one-way analysis of variance and Duncan's multiple range test. The following were observed:

(a) method of drying significantly affected the WHC of denatured whey protein powders. WHC of freeze dried powder was significantly higher than the others; WHC of air dried (25°C) powder was significantly lower than the others (both at $p \leq 0.01$).

(b) there was no significant difference between drum dried, spray dried, air dried (40°C) and vacuum dried powders ($p \leq 0.05$).

The initial WHC of the fresh curd was in the range 754-759 g water/100 g dry protein. Freeze drying retained approximately 75-80% of the original WHC whereas the other drying methods resulted in a 26-40% retention. An examination of the bulk densities of these products showed that drum dried, spray dried, air dried and vacuum dried powders had significantly higher bulk densities than freeze dried.

The surface geometry of each powder was examined by scanning electron microscopy (SEM) and the porosity was calculated from the sorption isotherms. These results will be discussed more fully in Chapter 5, but it is important to note the following:

(a) pore size distributions of freeze dried, spray dried, air dried and vacuum dried powders were very similar;

(b) the surface geometry of freeze dried powder and spray dried powder was very similar, each had an open porous structure Drum dried and air dried powders had similar flat, non-porous surfaces.

It is highly probable that there is a complex interrelationship

between surface geometry, internal geometry (porosity), bulk density and WHC. The surface geometry of the powders will determine the rate and extent of rehydration and it would be expected that the open porous structure of the freeze dried and spray dried powders would enhance penetration of water into the powder particles. The effect of porosity on WHC (section 5.2.5) is questionable. The bulk density of the powder will determine the extent to which each individual powder particle will be wetted.

This would seem to indicate that the bulk density, as influenced by both the composition of the powder and the drying method, is an important determinant of the WHC of denatured whey protein powders.

It is interesting to note that there was a satistically significant difference (at the 5% level of significance) between batch 4 and batch 5. With the exception of the spray dried powders, WHC tests on powders in batch 4 were significantly higher than those in batch 5. Both batches were dried under the same conditions and the proximate analyses of the batches were similar. It is possible that the differences in WHC are due to experimental error but, more probably, the differences are due to the inherent difficulties encountered when studying a biological material.

Each batch was produced from a different load of whey and, as such, the protein, carbohydrate, ion profiles may not be the same in all cases. Changes in milk storage conditions, cheese making conditions and storage and transportation of whey could have resulted in variations in the above mentioned profiles. Thus it could be expected that there would be different degrees of component interaction, when each batch of whey is heated. In particular, the interactions of importance may

be protein-lipid (Karel, 1973); protein-lactose (Webb and Johnson, 1965); protein-protein through cross-linkage of side chain amino acids (Friedman, 1977). The protein-lactose linkage has been reported to occur in whey systems (Webb and Johnson, 1965) and has been demonstrated to be a covalent linkage. An excellent review on protein-lipid interactions has been published by Karel (1973). Unfortunately, most protein-lipid interaction studies on dairy products are concerned with the interaction of protein and lipid at the fat globule surface in milk. The extent of protein-lipid interaction in whey has not been established although Short *et al.* (1978) noted that the lipid fraction in whey becomes associated with the insoluble protein fraction during processing. The importance of this interaction and the fraction of lipid which associates with the heated whey protein requires careful research.

To the author's knowledge, the nature or extent of protein crosslinking in heated whey systems has not been fully established. Crosslinked amino acids have been found in hydrolysates of heat treated proteins (Friedman, 1977). The mechanics of these crosslinkages have not been explained but it is thought that they are derived from "...addition of active hydrogen bearing protein function groups to the double bonds of dehydroalanine and...dehydroalanine residues could be derived from cystine, cysteine and serine side chains...." (Friedman, 1977). Because of the existence of cystine and serine side chains in α-lactalbumin and β-lactoglobulin (McKenzie, 1971; Smith, 1976), it can be expected that these linkages would occur in whey protein.

Because batches 4 and 5 had somewhat different protein contents (a difference of 2% could be significant), differences in the degree of crosslinking (if it occurs) could be used to partially explain the

observed difference in WHC.

An examination of table 7 shows that the bulk densities of freeze dried batches 4 and 5 were different; batch 4 had a lower bulk density than batch 5. Surface geometries and pore size distributions of these powders were essentially the same, so another probable explanation for the differences in WHC could be the difference in bulk density (as affected by difference in composition) of the freeze dried powders.

In the interests of retention of WHC, freeze drying appears to be the method of choice. This method is expensive and could nullify the objective of producing a cheap useable by-product from dairy wastes. Available equipment and cost of new equipment will determine which drying method would be chosen by a processor. In dairies that already own spray drying or roller drying equipment, these methods of dehydration would be the most economical choices. Further research should be undertaken to modify the bulk densities of spray dried and roller dried powders so that WHC could be increased.

Air drying cannot be overlooked as a potential industrial drying method for denatured whey protein curd. The WHC is comparable to spray dried or drum dried products and it would not involve a large capital outlay. A problem with this method is that the dried product forms very hard lumps and sheets. Secondary equipment would be required to produce a powder from the dry raw material.

4.3.3. Effect of Storage Conditions on WHC

The ability of a denatured whey protein powder to remain in an unchanged state during storage is important to both the manufacturer and user. To determine the effect of storage temperature and storage time on WHC of denatured whey protein powder, samples of freeze dried powder

Table 10: Effect of Storage Conditions on WHC of Denatured Whey Protein Powder (Batch 2)

Storage Time	Storage Temp. (°C)	WHC g water/100 g dry protein	
		5°C	25°C
0 days	-	754 ± 3.0	676 ± 4.5
100 days	5	742 ± 7.0	719 ± 13.0
	25	768 ± 9.5	705 ± 10.0
	40	781 ± 11.0	706 ± 11.0
200 days	5	748 ± 12.0	726 ± 3.0
	25	757 ± 8.0	699 ± 8.0
	40	781 ± 11.0	711 ± 15.0

from batch 2 were vacuum packaged into water impermeable plastic bags and stored at three different temperatures: 5°C, 25°C and 40°C. Powders were analysed for WHC at 100 and 200 days. Table 10 summarises the results of the WHC tests.

Statistical analysis of the results showed the following:

(a) test temperature had, once again, significantly affected the WHC result (discussed previously);

(b) storage temperature did not significantly affect the WHC;

(c) storage time had a small but statistically significant effect on WHC of denatured whey protein powders. The WHC increased slightly during storage.

The level of significance associated with the effect of storage time on WHC varied with test temperature. At a test temperature of 5°C, the effect of storage time was only slightly significant at the 5% level (experimental F value 7.02; $F_{0.05}^{2.4} = 6.94$) whereas, at a test temperature of 25°C, WHC differences were highly significant at the 1% level (experimental F value = 19.82; $F_{0.01}^{2.4} = 18.00$).

4.3.4. Remarks

The method of drying significantly affected the WHC of the powders. Freeze dried powders had a substantially higher WHC than air dried, drum dried, spray dried or vacuum dried. Values of WHC ranged from 190 g water/100 g dry protein (air dried #3) to 558 g water/100 g dry protein (freeze dried # 3). Because of this range, the method of drying chosen industrially would depend not only on the cost of drying, but also on the end use of the product. Where low water binding capacity is required (e.g. in baked goods) air drying, drum drying or spray drying would be suitable. If the degree of water binding required is high (e.g. in

intermediate moisture foods, pasta products), freeze drying would be chosen.

Water holding capacity tests of denatured whey protein powders indicate that the product should be classified as a high absorption powder.

Adverse conditions of storage (45°C for 200 days) did not affect the freeze dried powders. There was no loss in WHC and the powders were still free flowing after 200 days.

The WHC test temperature was shown to significantly affect the test result; thus, all future results should be reported along with the temperature so that valid [illegible]isons can be made.

The changes in the WHC of denatured whey protein powders are most likely due to physical effects. The bulk density of the powders (as affected by the powder composition and drying method) may be an important determinant of the final WHC. The WHC of the powders could be affected by the surface geometry which will determine the rate and extent of rehydration.

CHAPTER 5

WATER SORPTION STUDY

5.1 Introduction

The water sorption study was undertaken in an attempt to answer questions that arose upon completion of the water holding study. These included:

(i) would drying method affect the monolayer moisture content and, if so, could the monolayer value be related to the WHC of denatured whey protein powders?

(ii) could the porosity of the powders, as calculated from the moisture sorption isotherm, be used to explain differences in WHC?

(iii) could the moisture sorption isotherm be used to predict the WHC of denatured whey protein powders?

Adsorption of water vapor onto a solid is a surface related phenomenon depending on the number of active sites. The application of the BET equation to the isotherm data enables the calculation of the BET monolayer value which, for the purposes of this work, was chosen as a measure of the water binding capacity of denatured whey protein powders.

The addition of water over and above the monolayer value induces water-water interactions which eventually will result in a point of complete hydration.

From a theoretical point of view, further questions about water sorption in denatured whey protein were of interest:-

(i) would the isotherms of denatured whey protein powders be temperature dependent?

(ii) would denaturation of native whey protein affect the monolayer moisture content?

(iii) would the energetics of water binding be affected by the drying method?

To conclude the study of water sorption in denatured whey protein powders, the unfreezable water contents of freeze dried, drum dried, spray dried and undenatured protein powders were determined using differential scanning calorimetry. The purpose of this study was two-fold:-

(a) could there be a relationship between monolayer moisture content and unfreezable water content?

(b) would the drying method affect the unfreezable water contents of denatured whey protein powders?

An analysis and discussion of water sorption in denatured whey protein powders is presented in this chapter.

5.2 Methodology

In this section the preparation and analysis of sorption isotherms of denatured whey protein powders is explained. The mathematical examination of the isotherms enabled the determination of the following parameters:-

(a) BET monolayer values (section 5.2.2)

(b) BET surface areas (section 5.2.3)

(c) BET heats of sorption (section 5.2.4)

(d) porosity and pore size distributions (section 5.2.5).

5.2.1 Preparation of Sorption Isotherms

Sorption isotherms of 10 denatured whey protein powders and 3 control materials (dialysed denatured, undenatured whey protein and

Table 11: Materials Used for Sorption Isotherms

Material	Batch No.	Temperature (°C)
Freeze Dried Powder	1	25
Freeze Dried Powder	2	12, 25, 40
Freeze Dried Powder	3	12, 25, 40
Freeze Dried Powder	4	25
Freeze Dried Powder	5	25
Drum Dried Powder	3	12, 25, 40
Spray Dried Powder	3	12, 25, 40
Air Dried Powder	3	25
Vacuum Dried Powder	3	25
Undenatured dialysed whey protein	--	25
Denatured dialysed whey protein	--	25
Lactose Glass	--	25
Whey Protein Curd	4	25

lactose glass) were prepared using the evacuated dessicator technique. Samples of each powder were weighed into aluminum moisture dishes and vacuum dried for 48 hours at 60°C. After drying, the samples were placed in dessicators which were kept in a constant temperature chamber (Labline Inc., Chicago, Ill.). The dessicators were equilibrated to known water activities using various salt solutions as shown in table 12.

Table 12: Water Activities of Saturated Salt Solutions

Saturated Salt Solution	Water Activity (25°C)	Water Activity (40°C)
Lithium chloride	0.111	0.111
Potassium acetate	0.23	0.23
Magnesium chloride	0.329	0.31
Calcium nitrate	0.518	0.52
Sodium chloride	0.755	0.75
Potassium chromate	0.865	0.82
Distilled water	1.000	1.000

(values from Wink and Sears, 1950; Rockland, 1960)

Triplicate samples were placed into each dessicator and were weighed every 48 hours until "constant weight", i.e. no weight change at the third decimal when the sample was weighed to four decimal points. Adsorption equilibrium times for samples at 12°C were 21-26 days; 25°C 14 to 17 days; 40°C 9 to 12 days.

The desorption isotherms were prepared by equilibrating the samples in a 100% relative humidity dessicator. The samples were then transferred to dessicators of lower relative humidity until the new equilibrium was reached.

After equilibration, the moisture contents of each sample were determined by weight difference and the results were expressed in grams water per 100 grams of dry solids. The isotherms were prepared using the computer technique developed by LeMaguer (1977) as described in Appendix B. The monolayer moisture contents were determined using the BET method as outlined in section 5.2.2 (equations 5.1 and 5.2) and Appendix C. The BET surface area was calculated using equation 5.3 (section 5.2.3) and the BET thermodynamic constant (c) was obtained from the monolayer equation (Appendix C).

The net heat of adsorption (or BET heat of sorption) was calculated using equation 5.5 (section 5.2.4) and the heat of adsorption of the first layer of water molecules was calculated using equation 5.6.

5.2.2 BET Monolayer Value

The BET equation can be derived by a number of different methods which include statistical mechanics, reaction kinetics and thermodynamics (Labuza, 1968). The general BET equation is of the form:-

$$\frac{a}{(1-a)m} = \frac{1}{m_o c} + \frac{c-1}{m_o c}\, a \qquad [5.1]$$

where a = water activity

m = equilibrium moisture content g water/g dry solids

m_o = monolayer moisture content g water/g dry solids

c = thermodynamic constant

Equation 5.1 is a general linear equation of the form $y = a + b.x$. If the left hand side is plotted against the right hand side, a straight line will result with the intercept (I) equal to $\frac{1}{m_o c}$ and the slope (S) equal to $\frac{c-1}{m_o c}$. By rearranging the c term in the equations for I and S, it is possible to arrive at an equation for the monolayer moisture content (m_o).

$$m_o = \frac{1}{I + S} \qquad [5.2]$$

The BET equation only holds true for the lower part of the isotherm and, for denatured whey protein powders, above a water activity of 0.35-0.40 the graph produced by the equation departs from linearity.

Two important relationships can be obtained from the data after the monolayer value has been calculated. The first of these is the BET surface area of the product in question and, the second is the heat of water sorption in the product. The BET surface area is the total area available for water sorption and represents the area that is covered by the water monolayer.

5.2.3 BET Surface Area

By assuming the area of a water molecule to be 10.6 $\mathring{A}^2$ (Labuza, 1968), the BET surface area can be determined using equation 5.3:-

$$A = m_o \cdot \frac{1}{m_H} \cdot N. A_H \qquad [5.3]$$

where A = monolayer surface area (m^2/g solid)

A_H = surface area of one water molecule (10.6 $\mathring{A}^2$ = $10.6 \times 10^{-20} m^2$)

m_o = monolayer moisture content (g/g)

m_H = molecular weight of water = 18 g/mole

N = Avagadro's number = 6.02×10^{23} molecules/mole

5.2.4 BET Heat of Sorption

The thermodynamic constant (c) appearing in the BET equation can be expressed in the form:-

$$c = k.e^{(Q/RT)} \qquad [5.4]$$

where k = a constant value related to condensation coefficients and oscillation frequency of the adsorbed molecules (Gregg and Sing, 1967).

Q = net heat of adsorption

R = gas constant (0.41688 kJ/kg)

T = absolute temperature (°K)

Assuming that k approaches unity (Gregg and Sing, 1967), equation 5.4 can be rearranged to give:

$$Q = RT.\ln c \qquad [5.5]$$

The net heat of adsorption (Q) is the difference between the heat of adsorption of the first layer of the water molecules (E) and the latent heat of condensation of water (λ) at the temperature in question, i.e. $Q = E - \lambda$. Thus the heat of adsorption (E) can be simply calculated as:-

$$E = Q + \lambda \qquad [5.6]$$

Values for the latent heat of condensation used in equation 5.6 were; 2471 kJ/kg for 12°C, 2440 kJ/kg for 25°C and 2394 kJ/kg for 40°C.

5.2.5 Porosity and Pore Size Distribution

Another factor of importance when analysing water binding in foods is the porosity of the food. The porosity can be determined using the Kelvin equation (Gregg and Sing, 1967; Labuza, 1977):-

$$\ln a = \frac{-2\lambda V}{rRT} \cdot \cos \phi \qquad [5.7]$$

where a = water activity

λ = surface tension

V = molar water volume

ϕ = wetting angle

R = gas constant

T = absolute temperature

r = capillary radius

Equation 5.7 covers the case where the contact angle in the capillary is not zero but has a finite value ϕ. Before capillary condensation occurs, there is already a layer of adsorbed water on the walls. Conversely, during evaporation, there will be a layer of adsorbed water left behind on the walls. This is a fundamental assumption of the BET analysis. So, in effect, the application of the Kelvin equation (equation 5.7) is not measuring the pore radius (r) but the radius of the "smaller cylinder" that forms due to adsorption of water molecules on the pore surface. For the calculation of pore size distribution using the Kelvin equation [5.7], the thickness of the adsorbed layer (t) and the radius of the "smaller cylinder" (r_k), must be taken into account. It can be seen that;

$$r = r_k + t \qquad [5.8]$$

where r = true pore radius

The BET equation cannot be used to calculate the thickness of the adsorbed layer (t) over the whole isotherm. To calculate t, Gregg and Sing (1967) propose the use of Halsey equation:-

$$t = T \left[\frac{5}{\ln (P_o/P)} \right]^{1/3} \qquad [5.9]$$

where T = average thickness of a single layer of adsorbed molecules (4.3 Å).

In application of the Kelvin equation [5.7], it is assumed that the angle of contact is zero (Gregg and Sing, 1967). By substituting the value of the true pore radius (r) calculated using equation 5.8 into equation 5.7 and rearranging, we have:-

$$r_k = \frac{2\,\lambda V}{RT\ \ln a} - t \qquad [5.10]$$

The value r_k is known as the critical pore radius and is defined as the radius above which all pores are filled with adsorbed or condensed vapor (Gregg and Sing, 1967).

The pore radius (r), critical radius (r_k) and film thickness (t) for the pores in each powder sample were calculated using equations 5.7, 5.8, 5.9 and 5.10. The pore size distribution was calculated according to the method outlined in Gregg and Sing (1967). The pore size and pore size distribution of cylindrical and spherical pores were calculated for each powder using a computer program developed by Le Maguer (1977). The program provides an analysis of the pore parameters as well as graphs of the pore density (as a function of pore radius).

According to Gregg and Sing (1967), pores with widths less than 20 Å are classified as micropores, pores with widths greater than 200 Å as macropores and transitional pores (r = 20 Å – 200 Å) in between.

5.2.6 Unfreezable Water Content.

Kuprianoff (1958) suggested that the most accurate method to determine the extent to which water is "bound" in foods is the freezing

method. Basically, this method involves a determination of the amount of water which remains unfrozen below a specific temperature; usually below -40°C. The amount of "bound" water is considered to be equal to the unfrozen water; the free (or frozen) water can be determined calorimetrically.

One method of determining unfreezable water content that has been used by a number of workers is differential thermal analysis. Quantitative differential thermal analysis is called differential scanning calorimetry. Both methods measure the difference in heat absorption between a sample of the material to be studied and a reference sample; the measurement being conducted while both samples are subjected to the same thermal environment.

Unfreezable water contents in denatured whey protein powders were determined using a method developed by Duckworth (1971). Samples of freeze dried, spray dried, drum dried denatured protein powders and freeze dried undenatured protein were dried and placed in dessicators of known water activity (0.51, 0.68, 0.76, 0.88, 1.00) until equilibrium was reached. This preliminary equilibration was required to determine the range of water content at which free water could be detected by DSC. Following this, a second set of samples was placed into a 100% relative humidity dessicator. Samples were removed daily and the unfreezable water determination was carried out over a smaller range of moisture content.

The DSC analysis was performed using a Du Pont 900 Differential Thermal Analyser. After equilibration, 30-40 mg samples were weighed into aluminum cups, sealed, and placed into the test cells in the DSC apparatus. The cells were closed and both the sample and reference

material were frozen to -120°C using liquid nitrogen. Use of water-free protein powder (vacuum dried to remove residual moisture) as the reference sample eliminated the problem of base line slope. After freezing, the DSC cell was insulated and then allowed to warm to room temperature (25°C). The average warming rate was 30°C per hour. The difference in temperature (Δt) between the reference sample and test sample was measured and recorded using the temperature recorder on the Du Pont 900 DTA apparatus. The sensitivity used was 0.5 mV which corresponds to a 0.5°C/inch vertical linear scale. The machine was calibrated to 0°C using deionised, distilled water as the reference.

Samples of each powder were subjected to the procedure described. The presence of a peak at 0°C indicated that a phase change had occurred - water that was able to be frozen ("free" water) was present and had changed from a solid to a liquid state.

5.3 Results and Discussion

5.3.1 Effect of Dehydration on Water Binding in Fresh Denatured Whey Protein Curd.

To determine the effect of drying on WBC in denatured whey protein curd, a series of isotherms were produced using a fresh batch (# 4) of curd as the raw material. The curd was weighed into aluminum moisture dishes and a desorption isotherm was produced. After equilibration, all samples were weighed and left to dry in a 0% relative humidity dessicator. When drying was complete (10-14 days), an adsorption isotherm was constructed. The same samples were then subjected to a second ad-desorption cycle.

The results obtained showed conclusively that there was a significant loss in WBC and that equilibration of the dried product to the original

moisture level of the fresh curd did not occur. These isotherms (figures 3 and 4) show significant hysteresis down to zero water activity. The re-adsorption isotherms are almost superimposable but show a loss (about 30%) in WBC at saturation (a_w = 1.00) when compared to the initial adsorption curve. The loss in WBC upon drying was quite substantial. The fresh curd had an initial moisture content of 393.5 g water/100 g dry solids but, after desorption, the product would only equilibrate to 96 g water/100 g dry solids (at a_w = 1.00). The isotherms clearly demonstrate the non-reversibility of the desorption (i.e. drying) process.

The BET analysis shows the most convincing evidence for loss in WBC. The desorption and adsorption monolayer values (table 13) were 10.28 and 4.56 g water/100 g dry solids respectively. This would indicate that, during desorption (drying), there were a number of changes which reduced the total number of active sites available for readsorption of water. These changes could include:-

(a) shrinkage of the curd during dehydration with a resulting loss in total surface area available for readsorption of water. The BET surface area was reduced by over 50% when the wet curd was desorbed in the dessicators (BET surface area in the wet curd = 377 m^2/g; BET surface area in the dried curd = 160 m^2/g).

(b) component interactions (such as protein-protein or protein-ion) which lead to a loss in the number of active sites available for water readsorption and a tightening of the structure of the curd thus reducing the total surface area for readsorption.

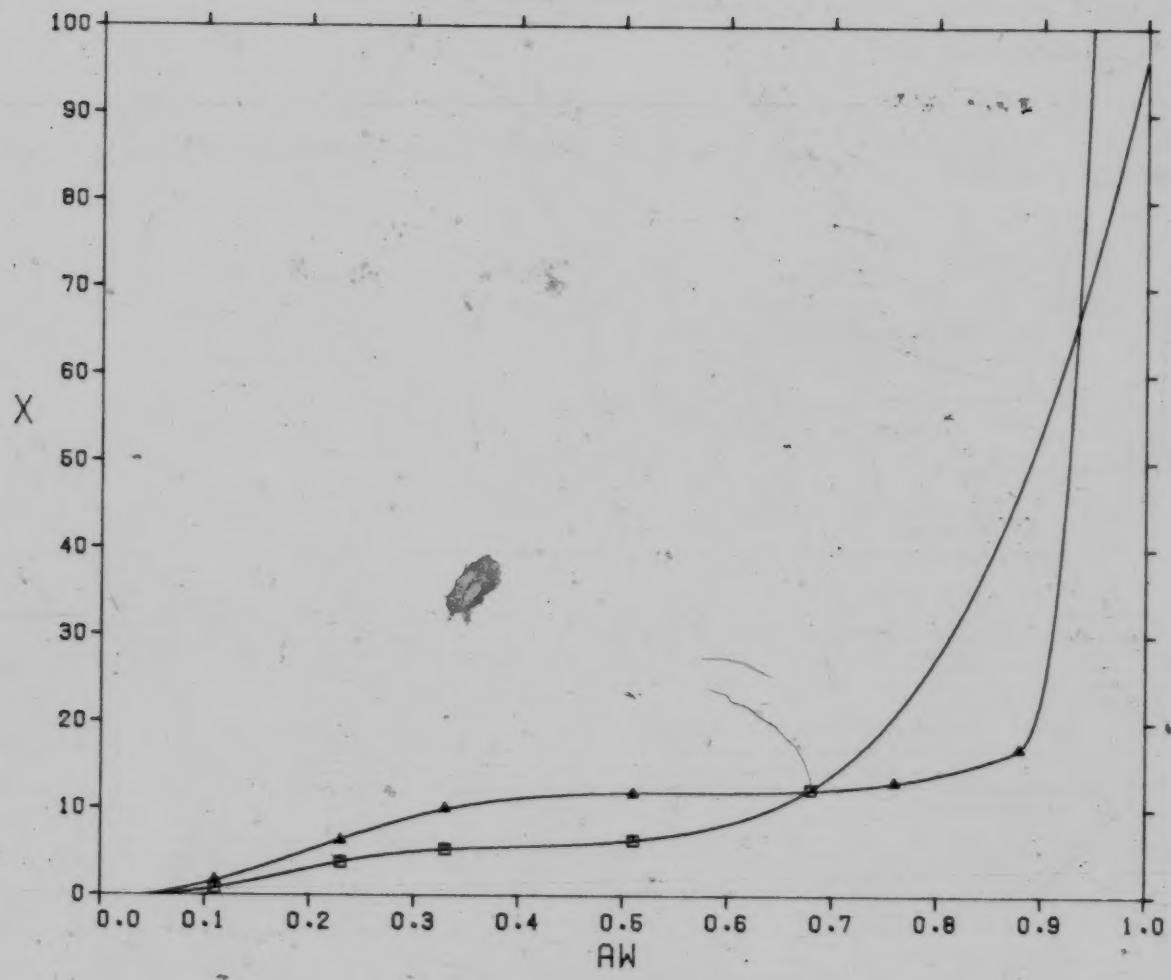


Figure 3: Desorption-Adsorption Isotherms of Denatured Whey Protein Curd (25°C)

Note: X = kg water/100 kg dry matter

□ = adsorption

Δ = desorption

initial moisture content = 3.935 g water/g dry solids.

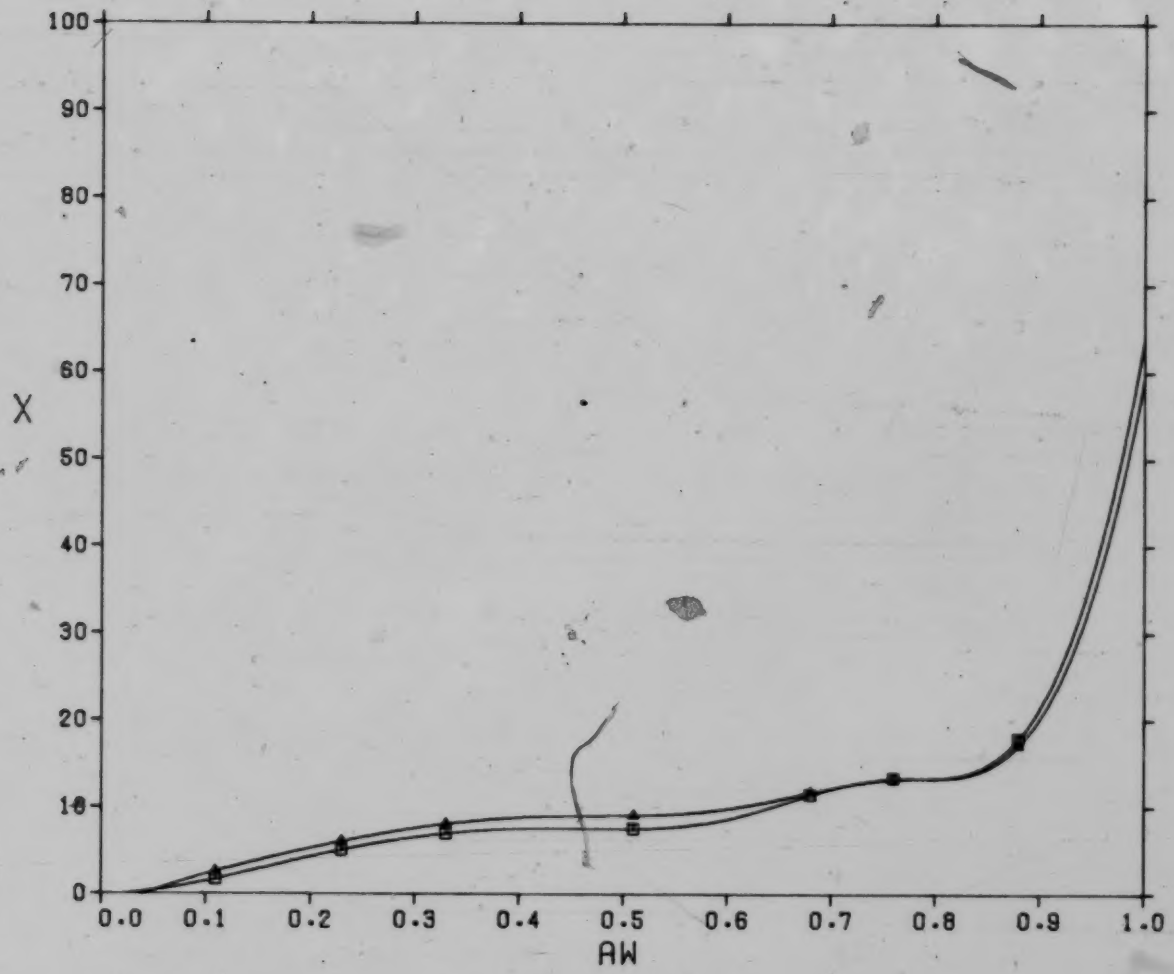


Figure 4: Desorption-Adsorption (second cycle) Isotherms of Denatured Whey Protein Curd (25°C)

Note: X = kg water/100 kg dry solids

□ = adsorption

Δ = desorption

Evidence for the possibility of component interaction in the wet curd is seen when comparing these analyses with those of the freeze dried powders. Physical shrinkage is minimised by the very nature of the freeze dehydration process yet the BET surface area was reduced by approximately 15% when compared to the wet curd. An extensive literature search has failed to reveal any evidence that protein-protein interactions occur in the wet curd. If they did occur, the interactions during drying (and rehydration) could be used to explain some of the observed differences in WBC. This area has to be extensively researched before any definite conclusions can be drawn.

Thermodynamic analysis of the data showed that the net heat of desorption for the protein curd was 146 kJ/kg whereas the net heat of adsorption was 246 kJ/kg. This seems to indicate that, in the wet curd, the water is weakly held with the thermodynamics favoring the desorption process. However, the significance of this result may be suspect due to the inherent inaccuracies in the BET method (refer section 5.3.5).

To conclude, experimental evidence suggests that physical changes in the protein curd are primarily responsible for the loss in WBC. It could be hypothesised that the bulk of the water is physically entrapped in the curd by a protein mesh. When the curd is dried, the physical structure of the curd is altered (the drying method determining the degree of alteration) and, therefore, all of the water removed cannot be reincorporated on rehydration.

5.3.2 Classification of Powder Isotherms

The adsorption-desorption isotherms of each powder and the pore size distribution graphs for the adsorption leg of the isotherm are all collected in Appendix D. The isotherms could be classified as either

type II or type IV according to the BET classification. With the exception of the isotherm for the dialysed, undenatured protein, there is a finite degree of adsorption as the saturation vapor pressure is approached. Thus, the isotherms may be more correctly classified as type IV isotherms. Type IV isotherms are usually associated with insoluble porous solids where internal adsorption is limited by the pore diameter.

The type IV isotherm has the normal sigmoidal shape of the type II isotherm but, more convincing evidence for the type IV classification is the presence of a hysteresis loop. Hysteresis loops can be found with other isotherm types (Gregg and Sing, 1967) but are more commonly associated with the type IV isotherm. All of the isotherms in Appendix D show some degree of hysteresis. The hysteresis loop usually ends at the a_w corresponding to the monolayer (Labuza, 1968) but, as can be seen in these isotherms, it can extend down to a water activity of zero. With the exception of the vacuum dried whey protein isotherm, all of the hysteresis loops extend into the monolayer region and approach zero water activity. The hysteresis loop for the vacuum dried whey protein ends at a water activity that approximates the monolayer water activity for this product. It is interesting to note that there is some degree of crossover between the adsorption and desorption branches of the isotherms. The significance of the crossover has not been established.

The lactose glass isotherm (Appendix D) does not resemble any of the traditional BET isotherms. Very little water was sorbed until a water activity of 0.92 at which point solubilisation commenced. The contribution of lactose glass (if present) to the WBC of denatured whey protein powders was insignificant.

As can be seen in Appendix D, isotherms of freeze dried batch 2, and batch 3, drum dried (# 3) and spray dried (# 3) were prepared at three different temperatures (12°, 25°, 40°C). The reason for this was twofold:-

(a) to determine the effect of temperature on the BET monolayer value (section 5.3.3);

(b) to apply the Clausius-Clapeyron equation so that the thermodynamics of the sorption process could be studied in detail (section 5.3.5).

5.3.3 BET Monolayer Values

Table 13 summarises the data obtained from the BET analysis of the isotherms of the powders dried by the five methods. There are two monolayer values reported - $m_o(A)$ and $m_o(B)$. Iglesias and Chirife (1976a) suggested that the validity of the BET monolayer value $\{m_o(A)\}$ was questionable in products that had low thermodynamic constants (c). These workers have shown that with a low value of c (less than 4-5), the shape of the isotherms digresses from the traditional type II or type IV isotherm (as evidenced with some of the whey protein powders showing low c values); the moisture content values (x) are very small and therefore of lower accuracy.

Even though 75% of the calculated c values for whey protein were above 4-5, the method of Iglesias and Chirife (1976a) was applied to the experimental data to test the validity of the BET theory as applied to denatured whey protein isotherms. To calculate the monolayer moisture content using this method, the water activity at which the monolayer occurs $\{a_w(m)\}$ had to be determined using the equation developed by these workers:-

Table 13: BET Values of Denatured Whey Protein Powders and Components

Material	Entry Number	Temp. (°C)	a_w (m)	m_o(A) g water/ 100 g dry solids	m_o(B)	% Diff.	c	BET Area (m^2/g)
Freeze Dried Batch 2	1	12	0.3082	6.17	6.20	0.49	5.04	218
	2	25	0.1824	4.73	4.85	2.54	20.09	167
	3	40	0.2199	2.06	2.08	0.97	12.58	73
Freeze Dried Batch 3	4	12	0.3405	9.05	9.05	0.0	3.75	317
	5	25	0.3209	8.73	8.66	0.80	4.48	306
	6	40	0.2604	5.58	5.80	3.94	8.07	195
Drum Dried Batch 3	7	12	0.3526	7.18	7.21	0.42	3.37	254
	8	25	0.2313	5.51	5.61	1.81	11.04	195
	9	40	0.2459	4.52	4.56	0.88	9.40	158
Spray Dried Batch 3	10	12	0.3405	9.25	9.28	0.32	3.75	324
	11	25	0.3125	8.62	8.60	0.23	4.84	302
	12	40	0.3253	7.51	7.39	1.60	4.30	263
Freeze Dried Batch 1	13	25	0.1575	4.53	4.52	0.22	28.63	160
Freeze Dried Batch 4	14	25	0.2377	6.64	6.36	4.22	10.28	232

Table 13 continued

Material	Entry Number	Temp. (°C)	a_w (m)	m_o(A) g water/ 100 g dry solids	m_o(B)	% Diff.	c	BET Area (m^2/g)
Freeze Dried Batch 5	15	25	0.2638	7.15	7.02	1.82	7.79	250
Air Dried Batch 3	16	25	0.3005	6.37	6.42	0.78	5.42	225
Vacuum Dried Batch 3	17	25	0.2671	6.94	6.90	0.58	7.53	243
Whey Protein Dialysed, Denatured	18	25	0.1711	9.03	8.92	1.22	23.46	316
Whey Protein Dialysed, Undenatured	19	25	0.2056	9.06	9.42	3.97	14.92	317
Lactose Glass	20	25	0.591	0.54	0.545	0.93	27.94	19
Whey Protein Curd; Adsorption	21	25	0.2707	4.56	4.66	2.19	7.26	160

Table 13 continued

Material	Entry Number	Temp. (°C)	a_w(m)	m_o(A) g water/ 100 g dry solids	m_o(R)	% Diff.	c	BET Area (m^2/g)
Whey Protein Curd; Desorption	22	25	0.3578	10.78	10.80	0.19	3.22	377
Whey Protein Curd; Readsorption desorption	23 24	25 25	0.2997 0.3175	6.43 7.92	6.54 7.97	1.71 0.63	5.46 4.62	225 277
Freeze Dried Batch 3;								
Replicate A Replicate B	25 26	25 25	0.3192 0.3192	8.71 8.71	8.59 8.64	1.26 0.80	4.55 4.55	304 304
Spray Dried Batch 3;								
Replicate A Replicate B	27 28	25 25	0.3116 0.3132	8.64 8.61	8.62 8.58	0.23 0.35	4.88 4.81	302 301
Freeze Dried Batch 3;								
Replicate A Replicate B	29 30	25 25	0.1785 0.1753	4.69 4.65	5.02 4.46	6.6 4.09	21.19 22.12	164 162

Table 13 continued

Material	Entry Number	Temp. (°C)	$a_w(m)$	$m_o(A)$ g water/ 100 g dry solids	$m_o(B)$	% Diff.	c	BET Area (m^2/g)
Drum Dried Batch 3; Replicate A	31	25	0.2328	5.57	5.66	1.62	10.86	195

Notes: (i) $m_o(A)$ = BET monolayer moisture content

(ii) $m_o(B)$ = monolayer moisture content as determined by method of Iglesias and Chirife (1976a)

(iii) c = BET thermodynamic constant

(iv) $a_w(m)$ = monolayer water activity

(v) % Diff. = difference between $m_o(A)$ and $m_o(B)$.

$$a_w(m) = \frac{-1 + \sqrt{c}}{c - 1} \qquad [5.11]$$

The monolayer value $\{m_o(B)\}$ corresponding to this water activity was then read off from the isotherm. The degree of difference between $m_o(A)$ and $m_o(B)$ was then used to determine the accuracy (and validity) of the BET theory as applied to the isotherms studied. The degree of difference was largely subjective. As can be seen from table 13, the calculations of $m_o(A)$ and $m_o(B)$ show excellent agreement with the majority of values showing a difference of less than 2%. The BET monolayer values calculated for the isotherms can thus be considered reliable.

As can be seen in table 13, as the temperature was increased the monolayer moisture content decreased. This was first observed by Brunauer *et al.* (1938) and subsequently confirmed for most foodstuffs (Iglesias and Chirife, 1976a). This decrease in the BET monolayer value with an increase in temperature was originally thought to be due to the expansion of water molecules due to an increase in temperature (Brunauer *et al.*, 1938). Iglesias and Chirife (1976a) showed that this alone could not cause the demonstrated decrease in the monolayer value. These workers attribute the decrease to a reduction in the number of active sites available for water adsorption as a result of physical and/or chemical changes induced by temperature.

No significant difference was observed between denatured protein and undenatured protein with respect to their BET monolayer values and BET surface areas (entry numbers 18, 19). However there was a large difference in c and this points to the fact that changes in the number and/or activity of water sorption sites has occurred. An

examination of the isotherms in Appendix D shows that the denatured protein exhibits more hysteresis than the undenatured protein. Up to a_w = 0.35, the adsorption isotherms are similar, but between 0.35 and 0.85 the denatured protein adsorbs more water than the undenatured protein. Above a water activity of 0.90, the undenatured protein adsorbs more water than the denatured protein--this is obviously a solubility effect.

The shape of the undenatured protein isotherm resembles the more traditional type II isotherm for soluble materials as described by Gregg and Sing (1967). The BET monolayer values calculated from the isotherms were 9.03 g water/100 g dry solids (denatured protein) and 9.06 g water/100 g dry solids (undenatured protein). An examination of the undenatured and denatured protein isotherms and the BET monolayer moisture contents shows that thermal denaturation has little (if any) effect on the ability of whey proteins to absorb water from the vapor phase, confirming the work of Berlin *et al.* (1973).

Table 13 shows that the method of drying affects the monolayer moisture content of denatured whey protein powders. Freeze dried and spray dried powders (entry numbers 5, 11) had significantly higher monolayer values (8.73 and 8.62 g water/100 g dry solids) than the drum dried (#8), air dried (#16) and vacuum dried powders (#17) which had 5.51, 6.37 and 6.94 g water/100 g dry solids respectively at 25°C. Considering the fact that all of these products had similar protein contents (75-79% protein on a dry basis), the differences in the monolayer moisture content could be attributed to three factors:

(i) the extent of component interaction could be affected by the drying method. It is highly probable that the protein components of the curd could interact during drying and the

degree of interaction could depend on the drying method (section 2.2.3). Interaction of components would lower the number of active sites available for water sorption; as a result, the monolayer moisture content will decrease.

(ii) changes in external geometries as affected by the drying method. Scanning electron micrographs (figures E.1, E.2, E.3 and E.4) of drum dried, freeze dried, spray dried and air dried powders revealed marked differences in their surface characteristics. The freeze dried denatured whey protein powder (fig. E.1) had a very open, porous structure with a large number of voids. The spray dried powder (fig. E.2) consisted of a large number of porous spheres--a shape which is ideal for surface water adsorption because of the large surface area:volume ratio. The drum dried and air dried powders (figures E.3 and E.4) had very similar compact smooth surfaces.

(iii) changes in internal geometry (section 5.3.4). Freeze dried and spray dried powders had a greater pore volume than drum dried powders (e.g. at 25°C; freeze dried and spray dried powders had 30% of pores with radii less than 30 Å whereas the drum dried powder had only 20% of pores with radii < 30 Å). Pore size distributions of freeze dried and spray dried powders were nearly identical whereas the drum dried powder had a significant proportion of large pores. The freeze dried and spray dried powders have more surface area available for sorption and will, therefore, have larger monolayer values than drum dried powder.

With respect to freeze dried, spray dried and drum dried powders (entry numbers 5, 8, 11), porosity may be used to explain differences in WBC, but the effect of porosity on monolayer moisture content is not a general effect as explained in the next section.

5.3.4. Pore Size Distribution and Surface Geometry

Appendix D contains the pore size distribution graphs of all the denatured whey protein powders. Approximately 30% of the pores in the freeze dried (#3) and spray dried (#3) powders had a pore radius < 30 Å, whereas only 16-20% of the pores in the drum dried, air dried and vacuum dried products had pore radii < 30 Å. Powders with a large proportion of small radius pores could have a higher monolayer value than powders with a small proportion of small radius pores because of the increased surface area afforded by a larger number of smaller pores.

Closer examination of the pore size distribution graphs revealed that this pore size effect was not entirely applicable because of the following conflicting results:

(i) freeze dried batch 2 and freeze dried batch 3 had similar pore size distributions but different monolayer moisture contents (4.73 and 8.73 g water/g dry solids respectively);

(ii) freeze dried batch 4 and 5 had 50% of pores < 30 Å in radius and monolayer moisture contents of 6.64 and 7.15 g water/100 g dry solids. Freeze dried batch 3 (30% of pores with radius < 30 Å) had a monolayer moisture content of 8.73 g water/100 g dry solids.

There is no clear cut relationship between monolayer moisture content and porosity for denatured whey protein powders. It is obvious that the porosity of the product will affect the rate and extent of

rehydration of a dry food, but in the case of denatured whey protein powders, other factors must be taken into account which may explain why no precise relationship seems to exist.

Proteins have an unusual ability to bind water and the extent to which proteins bind water is dependent on the pH--as the pH is increased, the uptake of water increases (Hermansson, 1972). This reduction in the ability of proteins to bind water at acidic pH values could be attributed to a reduction in the number of active sites available for water adsorption due to protonation of those sites. In a 6% w/w suspension, the pH values of freeze dried batch 1, 2 and 3 were 5.21, 4.84 and 6.51 respectively. This could explain why, even though the powders from all of the above batches had similar porosities, the water adsorption ability of the proteins in batch 3 was higher than the water adsorption ability of the proteins in batches 1 and 2.

The second important factor which may affect any possible relationship between porosity and the water binding capacity is the surface geometry of the powder. No matter how porous the internal surface of the product is, if the surface geometry precludes entry of the water vapor into the internal surface of the powder, the powder in question will likely have a low water binding capacity.

The porous surface features of the freeze dried and spray dried powders (figures E.1, E.2) and the similarities in internal microstructure (porosity), protein content and pH may explain why freeze dried (batch 3) and spray dried (batch 3) had similar BET characteristics (monolayer and surface area). But the water holding capacities of these powders were different and this difference could be attributed to the only physical parameter that varied between the products--the bulk

density. The bulk density of the freeze dried powder was lower than the bulk density of the spray dried powder. The lower bulk density of the freeze dried powder means a looser packing of the particles and, therefore, the total quantity of water that will penetrate into the freeze dried powder mass will be greater than the spray dried powder (higher bulk density, tighter packing of particles).

The drum dried and air dried powders had similar smooth surface features (figures E.3, E.4) but the air dried powder had a greater proportion of pores with a radius < 30 Å. Protein content and pH of each powder were similar (protein 76-78%, pH 6.5) but the monolayer moisture contents were different (5.51 g water/100 g dry solids for the drum dried powder and 6.37 g water/100 g dry solids for the air dried powder). In the case of air dried and drum dried powders, the difference in porosity could explain why the monolayer moisture contents are different.

There appears to be no simple relationship between the WBC, porosity and surface geometry of denatured whey protein powders. It has been shown that the drying method affects the WBC of the powders, freeze drying having the least affect on WBC. Evidence has been presented to indicate that porosity, surface geometry and changes in the number of active sites may affect the water binding capacities of the powders. Individual effects and interactions between the factors are unknown at the present time.

When referring to drying, care has to be taken when discussing the physical effect of the structure on adsorption. Because water is such a small molecule, it is only in the micropore region that structural (or porosity) differences could be significant. Considering the fact that approximately 95% of the pores in all powders fall within the

intermediate or macropore classification, changes in porosity per se may have little or no effect on water binding. Differences in micropore structure between powders could lead to changes in the number or distribution of water sorption sites and this change will have an obvious effect on the WBC as measured by the BET monolayer method.

5.3.5 Thermodynamic Analysis of Powders

No discussion on the water binding capacity of food products is complete without an analysis of the thermodynamics of the system. Table 14 summarises the net heat of adsorption (Q) and the heat of adsorption (E) of water onto the surface of the powder. Both Q and E apply to the first layer of molecules only. The value of Q represents the heat released due to site interaction only, whereas E includes this value plus the latent heat of condensation that is released when the water molecule condenses at the active site.

Equation 5.4 was used to calculate Q. To simplify this equation the value of k is often taken as 1 but Iglesias and Chirife (1976b) point out that this constant may vary over a very wide range and that "the value of C constant cannot be taken as any more than a rough guide to the value of Q; this is especially true when dealing with sorption of water on food systems." Iglesias and Chirife (1976b) note that the heat of sorption estimated by the BET method is often significantly lower than the actual heat of sorption.

Because of the possibility of error in the BET analysis, an alternative method of calculating the thermodynamic constants is the Clausius-Clapeyron equation. This equation is a differential quantity which varies with the degree of surface coverage; hence the isotherms produced at different temperatures.

Table 14: C Values, Net Heats of Adsorption and B.E.T. Heats of Adsorption for Whey Protein Powders and Components

Description	Temp.	C.	Q Net heat of adsorption (kJ/kg)	Q + λ Heat of adsorption (E) (kJ/kg)
Freeze Dried Batch 2	12	5.04	184	2655
	25	20.09	373	2813
	40	12.58	330	2724
Freeze Dried Batch 3	12	3.75	150	2621
	25	4.48	186	2626
	40	8.07	272	2666
Spray Dried Batch 3	12	3.75	150	2621
	25	4.84	196	2636
	40	4.30	190	2584
Drum Dried Batch 3	12	3.37	138	2609
	25	11.04	298	2738
	40	9.40	292	2686
Freeze Dried (Batch 1)	25	28.63	417	2857
Freeze Dried (Batch 4)	25	10.58	289	2729
Freeze Dried (Batch 5)	25	7.79	255	2695
Air Dried (Batch 3)	25	5.42	210	2650
Vacuum Dried (Batch 3)	25	7.53	251	2691
Whey protein (den.)	25	10.28	282	2727
Whey protein (unden.)	25	7.79	255	2695
Lactose glass	25	27.94	414	2854
Protein curd (3.935 H_2O/g solids)	25	7.26	246	2686
Protein curd (readsorption)	25	5.46	211	2651
Protein curd (des.)	25	3.22	146	2586

The application of the Clausius-Clapeyron equation to freeze dried batches 2, 3, drum dried batch 3 and spray dried batch 3 isotherms--in an attempt to produce an isoteric heat plot--proved unsuccessful. These isotherms were virtually superimposable at the temperatures studied (12°C, 25°C, 40°C) with only small differences between individual isotherms. With the exception of the monolayer region (a_w 0.10-0.35), it could be concluded that isotherms of denatured whey protein powders were non-temperature dependent.

An extensive literature search failed to show whether non-temperature dependence was a general phenomenon. Berlin *et al.* (1970) were able to produce isoteric heat plots of spray dried skim milk and freeze dried undenatured whey powder (low protein). However, Kuntz and Kauzmann (1974) state that, with regard to proteins, "...isotherms at various temperatures are virtually superimposable for nitrogen adsorption, and no hysteresis is observed."

When the Clausius-Clapeyron equation cannot be used to produce an isoteric heat plot because of superimposability of isotherms, the BET method is the only "reliable" estimate of the heat of water sorption in denatured whey protein powders.

Because the results shown in table 14 are confined to the monolayer region, they yield only a small (but significant) amount of information on the energetics of water sorption in denatured whey protein powders. There were some notable differences in the net heats of adsorption (Q) between denatured whey protein powders dried under different conditions.

The value of Q is an indication of the strength of adsorption at the active sites. The data indicate that temperature affected the Q

value; Q decreased with decreasing temperature. The values of Q depend on a number of factors which include the degree of swelling of the adsorbent, the number of polar groups on the adsorbent, the extent of hydrogen bonding between the adsorbed material and the adsorbent. The heat of swelling is highly endothermic and may use as much as 50% of the heat produced by the adsorption process.

Q values for freeze dried, spray dried, air dried, vacuum dried and drum dried batch 3 (186, 196, 210, 251, 298 kJ/kg at 25°C respectively) indicate that the method of drying may affect the way in which water is bound to the substrate. The differences in Q could be related to the number of active sites available for water sorption. The trend observed with the monolayer values and BET surface areas continues with the Q values. These three variables present fairly strong evidence for the occurrence of component crosslinking and that the degree of crosslinking may be greater in powders produced by slow drying (air drying, vacuum drying, drum drying). Obviously the freeze dried powder is slow dried but it is an exception to the rule because the water is immobilised and the chance of component crosslinking is minimised.

The thermodynamic values obtained are only estimates of the actual values. They could be substantially lower than the "real" values (Iglesias and Chirife, 1976b; Mazza and LeMaguer, 1978) and, because of this, less emphasis should be placed on the quantitative aspects of these results. They cannot indicate the mechanism by which water is bound in these powders or at what sites the binding occurs. Qualitatively, though, they do give an indication of the strength of the bond and this may be related to the way in which water is bound in the powders.

5.3.6 Accuracy of Dessicator Method for Producing Water Sorption Isotherms of Denatured Whey Protein Powders

All the isotherms were produced by averaging triplicate determinations of moisture content after equilibration at a specific relative humidity. Even though the standard error was, in most cases, less than 2%, this does not guarantee that the method is fully reproducible as the final equilibrium was not absolute.

To determine the reproducibility, triplicate isotherms of freeze dried batch 2 and 3, drum dried batch 3 and spray dried batch 3 were produced and analysed. Experimental data points for these isotherms are in Appendix 1 and the BET analyses are included in table 13 (entries 25-31).

In all of the replicate isotherms, the standard error for individual moisture contents was less than 1% and the monolayer values and BET surface areas were in excellent agreement. Statistical analysis of the results using a two-way analysis of variance computer program showed no significant difference between replicates at the 1% level of significance. As a result the method used for producing ad-desorption isotherms of denatured whey protein powders can be considered to be reliable and accurate for the purpose of this work.

5.3.7 Unfreezable Water Content of Denatured Whey Protein Powders

The method outlined in section 5.2.6 was used to determine the unfreezable water content of selected whey protein powders. Figure 5 shows a series of DSC scans obtained for a freeze dried denatured whey protein powder (batch 3). Curves for drum dried and spray dried powders were similar. The unfreezable water contents of each powder are summarised in table 15.

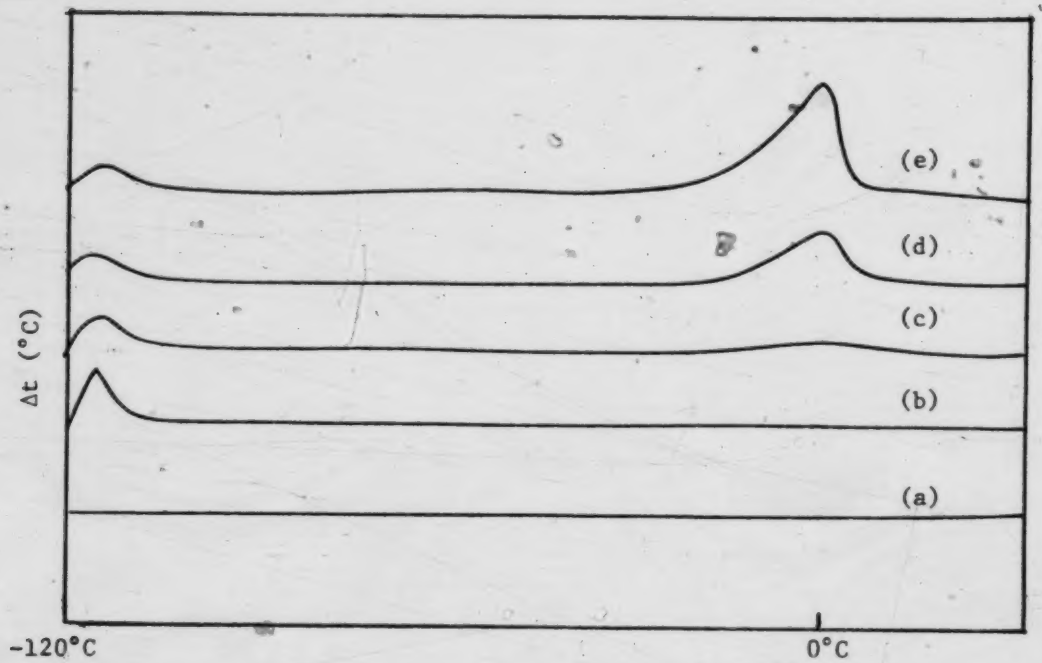


Figure 5: DSC scans of freeze dried denatured whey protein powder (batch 3).

(a) Baseline

(b) .4701 g water/g dry solids

(c) .4780 g water/g dry solids

(d) .4816 g water/g dry solids

(e) .4903 g water/g dry solids

Table 15: Unfreezable Water Content of Whey Protein Powders

Material	Unfreezable Water g H_2O/g dry solids
Freeze Dried # 3	0.4780
Drum Dried # 3	0.4138
Spray Dried # 3	0.4410
Undenatured dialysed whey protein	0.5138
Denatured dialysed whey protein	0.4706

Heat denaturation of whey proteins seemed to reduce the unfreezable water content when compared to the native protein. There is conflicting evidence as to the effect of denaturation on unfreezable water content. Various workers have shown decreases in the range 10-15% (summarised by Simatos *et al.*, 1975) whereas others (Berlin *et al.*, 1973) have shown that denaturation has no effect at all on the unfreezable water content. In this study, denaturation appeared to reduce the unfreezable water content by 8.5%.

The method of drying may affect the unfreezable water content. The unfreezable water contents of the freeze dried, drum dried and spray dried powders were reduced by 8%, 20% and 14% respectively. The differences could be related to the way in which water is bound in these powders. Table 16 summarises the monolayer moisture contents and unfreezable water contents of the powders.

There is no definite relationship between monolayer moisture content and unfreezable water content of denatured whey protein but a trend does exist. As the monolayer value decreased, the unfreezable water content decreased.

The inconclusive results given by the unfreezable water analyses are not surprising. In 1975, Simatos *et al.* stated "...it does not appear possible to draw any consistent relationship between the data (unfreezable water) and the chemical composition of the materials. This fact may be due to differences in techniques used. More probably, unfreezable water is not very specific to the chemical nature of the substrate.... Comparisons would be much more valid were it possible to take into account the physico-chemical properties of the substrate."

This may be the direction to take in any future studies on the

Table 16: Monolayer Moisture Contents and Unfreezable Water Contents of Denatured Whey Protein Powders

Test Material	Monolayer moisture content g H_2O/g dry solids	Unfreezable water content g H_2O/g dry solids
Undenatured Whey Protein	0.0906	0.5138
Denatured Whey Protein	0.0903	0.4706
Freeze Dried # 3	0.0873	0.4780
Spray Dried # 3	0.0862	0.4410
Drum Dried # 3	0.0551	0.4138

unfreezable water content of denatured whey protein powders.

5.3.7 Remarks

Water sorption studies of denatured whey protein powders have shown the following:

(a) the monolayer moisture content decreased with temperature;

(b) denaturation of native whey protein had no effect on the ability of the powder to sorb water from the vapor phase (at low a_w values). Denaturation reduced the unfreezable water content of whey proteins by 8.5%;

(c) the monolayer moisture contents and the net heats of adsorption were affected by the drying method. This effect could be related to changes in number of active sites available for water sorption;

(d) the processing method seemed to affect the unfreezable water content of denatured whey protein powders;

(e) there is a possibility of a relationship between the monolayer moisture content (m_o) and the unfreezable water content as determined by DSC. As m_o decreased, the unfreezable water content decreased.

The changes in the monolayer moisture contents could not be related to changes in the powder porosity per se. Rehydration of proteins is a dynamic process and interactions induced by hydration could change the character of the substrate. These interactions could include protein-protein, protein-ion and protein-carbohydrate. Probable changes in the number, type and activity of water sorption sites in the micropore region of the powders in conjunction with changes in surface and internal structure due to the drying method may be the cause of variation in

the BET monolayer values.

The relationship between the water sorption study and the water holding study will be discussed in the next chapter.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Relationship Between Water Sorption Study and Water Holding Study

Few studies have been reported on the relationships between sorption of water and functional properties depending on water-protein interactions, such as water holding capacity. To see if water sorption isotherms could be used to predict WHC of denatured whey protein powders, WHC and WBC results from chapters 4 and 5 were compared. Appendix D contains the moisture sorption isotherms and tables 9 and 13 summarise the WHC and WBC results.

Abhayaratna (1977, unpublished results) tried to relate data from water sorption isotherms to the water absorptive capacity of undenatured whey protein concentrates. His results showed that there was no relationship between the monolayer content and water absorptive capacity but water sorption in the higher regions of the isotherm did correlate with water absorption as determined by the farinograph method.

Table 17 is a summary of BET monolayer values and WHC tests for denatured whey protein powders. All powders were prepared from the same batch (no. 3). These data indicate that there is no strong relationship between monolayer moisture content and WHC although the WHC seems to decrease as the monolayer value decreases. Even though it appears as though the monolayer moisture content cannot be used to predict WHC, a moisture sorption study could still be useful. A more exhaustive analysis of the higher regions of the isotherm could show some relationship between water absorption and WHC.

Tables 18 and 19 summarise the data obtained for WHC (25°C) and

Table 17: BET Monolayer Values and WHC of Denatured Whey Protein Powders (Batch 3)

Drying Method	BET Monolayer Value (25°C) g water/100 g dry solids	WHC (25°C) g water/100 g dry protein
Freeze Dried	8.73	539
Spray Dried	8.62	237
Vacuum Dried	6.94	248
Air Dried	6.37	183
Drum Dried	5.51	244

Table 18: Water Sorbed at A_w = 1.00 and WHC of Denatured Whey Protein Powders

Batch No.	% Protein	% Lactose	[a]Water Sorbed (a_w = 1.00)	[b] WHC
1	64.81	29.41	117.10	659
2	69.06	25.03	64.24	676
3	78.01	16.65	32.74	539
4	91.68	5.83	35.29	587
5	90.37	6.79	31.54	545

(a) g water/100 g dry solids
(b) g water/100 g dry protein

Table 19: Water Sorbed at A_w = 1.00 and WHC of Denatured Whey Protein Powders (Batch No. 3)

Drying Method	Water Sorbed [a] (a_w = 1.00)	WHC[b]
Freeze Dried	32.74	539
Vacuum Dried	65.67	248
Drum Dried	61.83	244
Air Dried	59.07	183
Spray Dried	32.75	237

(a) g water/100 g dry solids
(b) g water/100 g dry protein

water sorbed (a_w = 1.00) for several denatured whey protein powders. It would seem that water sorption isotherms would have limited use (if any) for predicting WHC of denatured whey protein powders. As an example, drum dried (#3), vacuum dried (#3) and freeze dried (#2) powders all sorbed similar amounts of water at a_w = 1.00 but the results of the WHC tests were very different (244, 248 and 676 g water/100 g dry protein respectively). Similarly freeze dried (#3) and spray dried (#3) powders had the same amount of water sorbed at a_w = 1.00, similar protein and lactose contents but different water holding capacities (237 and 545 g water/100 g dry protein). These powders also had essentially the same pore size distributions, similar external surface geometries and the same pH (6.5). The only physical parameter that varied with the two powders was the bulk density (freeze dried bulk density = 0.41 g/cm^3, spray dried bulk density = 0.53 g/cm^3). This would suggest that, when all other factors are constant, the bulk density of a powder (as affected by the drying method) is a critical factor in determining the WHC of a denatured whey protein powder.

To summarise, the water sorption data seemed to have limited value for predicting the WHC of denatured whey protein powders. However, the water sorption study was extremely useful in substantiating the observed differences in drying methods, the effects of denaturation on water binding and changes in water sorption properties (e.g., the monolayer value or BET heat of sorption) as they relate to WBC.

6.2 Conclusions

Water sorption studies showed that the monolayer moisture content of denatured whey protein powders decreased with increasing temperature. This decrease in m_o is thought to be due to a reduction in the number

of active sites available for water adsorption due to physical and/or chemical changes induced by temperature (Iglesias and Chirife, 1976a). Chemical changes at the active sites could account for observed differences between batches dried by the same method. For example, the pH values for batch 2 and 3 were 4.84 and 6.51 respectively and the monolayer values were 4.73 and 8.73 g water/100 g dry solids. The lower pH of batch 2 could induce protonation of the carboxyl groups thus reducing the number of active sites available for water sorption.

The results also showed that the monolayer moisture content was affected by the drying method. The m_o of the fresh curd was 10.78 g water/100 g dry solids whereas the m_o values of freeze dried (#3), spray dried and drum dried powders were 8.73, 8.62 and 5.51 g water/100 g dry solids respectively. Freeze drying produced the smallest loss in m_o and drum drying the largest. Sorption studies showed that, protein was the most significant contributor to water binding below a water activity of 0.92. Lactose (if present) did not play a part in water adsorption until a water activity greater than 0.92

A pore distribution analysis showed that there was no discernible relationship between porosity and monolayer moisture content or porosity and WHC. The inability to relate pore size to these values could be attributed to swelling of the proteins as they hydrate. The swelling effect can be quite substantial after the initial layer of water molecules has been sorbed. The degree of swelling will vary with pH and the amount of protein -- both of these factors vary between the powders studied. The swelling of the proteins could negate any pore size effects.

The pore size distribution per se may not be as important in determining WBC/WHC as the number, activity and availability of water sorption sites in the micropore region. The distribution of pores as well as sorption sites in the micropore region could be affected by the method of drying. Because of the very nature of the process, freeze drying would not affect the porosity and number of active sites as compared to a severe drying process such as drum drying.

A comparison of the surface geometry by SEM showed that freeze dried and spray dried powders had very similar porous surface features. Drum dried and air dried powders had smooth surfaces with very few voids. The spherical, porous surface of the spray dried product and the porous cracked surface of the freeze dried product would allow rapid initial moisture penetration into the powder thus explaining the similar WBC of these powders. But the higher bulk density of the spray dried product means a tighter packing of the particles; therefore, the total quantity of water that penetrates into the spray dried powder mass will be less than the freeze dried powder (lower bulk density). This difference in bulk density could explain the difference in WHC of these powders. The drum dried and air dried powders would have much slower penetration rates (because of their surface features) thus producing surface protein swelling resulting in reduced water sorption to the interior and reduced WBC and WHC. It was noted during the WHC tests, that the interiors of individual air dried particles were dry, while the surface appeared fully rehydrated.

Convincing evidence for loss in WBC and WHC was provided by the analysis of a series of ad-desorption isotherms of fresh protein curd. Neither the monolayer moisture content nor the amount of water sorbed

at a_w = 1.00 returned to the initial pre-drying level. The monolayer value (m_o) and equilibrium moisture content of the fresh curd were 10.78 and 393.5 g water/100 g dry solids respectively. After dehydration, the m_o and water sorbed at saturation (a_w = 1.00) were 4.56 and 96 g water/100 g dry solids.

The energetics of the sorption processes were studied using the BET thermodynamic constant. As pointed out by Iglesias and Chirife (1976b), the BET method is not as accurate as the Clausius-Clapeyron method when used to determine heats of sorption. Because the isotherms showed little or no temperature dependence, it was not possible to produce a series of isoteres from which an isoteric heat curve could be constructed. Even accounting for the inaccuracies of this method, it provided valuable comparative data:

(a) with reference to the wet curd, the energetics of sorption favored the desorption leg of the isotherm; less energy was required to remove the water than to readsorb it;

(b) freeze dried and spray dried powders had lower net heats of adsorption than drum dried powders. This would indicate that the method of drying may affect the way in which water is bound to the substrate. It could also reflect the number and availability of water sorption sites.

WHC tests indicated that the drying method substantially affected the WHC of denatured whey protein powders. Freeze drying resulted in only a 20% loss in WHC but drum drying, air drying, spray drying and vacuum drying caused 70-80% loss in WHC. Low bulk density powders (freeze dried) had the highest WHC.

To summarise, the results of this work indicate that physical

changes during dehydration may be the main cause of the loss in WHC of denatured whey protein powders. The bulk density of the powder (as affected by the drying method and powder composition) is an important determinant of WHC. It could be hypothesized that the bulk of the water is physically held in the fresh curd by a compact protein mesh. In the case of spray drying, air drying, drum drying and vacuum drying, the physical structure could be irreversibly destroyed and, therefore, all of the water removed may not be reincorporated on rehydration. By the very nature of the process, freeze drying preserves the structural integrity of the curd thus allowing more complete rehydration.

From the analyses performed, it could be concluded that the loss in WBC and WHC may be the result of a number of factors which include:

(a) destruction of the macrostructure of the curd upon dehydration;

(b) alteration of the microstructure of the curd due to component interactions (the possibility of protein-protein or protein-ion linkages cannot be neglected);

(c) reduction and alteration in the surface area available for rehydration as shown by the SEM examination and change in BET surface area;

(d) reduction in the number of active sites available for water sorption due to differences in composition, microstructure and degree of component interaction.

6.3 Recommendations for Future Work

Although this work covers a lot of ground, there is still a large number of unanswered questions about loss in WHC upon dehydration of heat-acid coagulated whey protein curd. Several areas of investigation

are suggested as a possible means to further elucidate why this loss occurs:

(a) an extensive investigation of component interactions (i.e., protein-lipid, protein-protein, protein-ion, protein-lactose) using model systems;

(b) an investigation of component interactions during the heating of whey; a study of the heat-acid coagulation process using differential thermal analysis, ultracentrifugal analysis, circular dichroism or similar techniques may show any structural rearrangements that occur during heating;

(c) direct calorimetric measurement of the enthalpies and entropies of water adsorption in order to elucidate any changes that may occur in the binding of water on powders dried by different methods.

From an industrial point of view, the spray drying or drum drying methods could be modified so that low bulk density powders are produced. Methods such as foam spray drying, vacuum drum drying and explosion puff drying may have potential.

At the present time, the heat-acid coagulation process is only being used to its full potential by a few companies in various countries (notably New Zealand and Holland). The process is cheap (i.e. it requires very little capital outlay compared to other methods of producing whey protein concentrates such as ultrafiltration) and provides a highly nutritious, easy to use, high protein product. The method should be considered as a serious alternative to the problem of whey disposal in countries such as the U.S.A., Canada and Australia. Because the method has not yet reached universal acceptance, considerable

research effort should be directed towards maximising yield, minimising cost, improving functional properties and utilising both the curd and the supernatant after the heat coagulable protein has been removed.

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APPENDIX A

ISOTHERM EXPERIMENTAL DATA POINTS

The table included in the appendix is a summary of the experimentally obtained sorption isotherm data. Each sample was placed in triplicate in the dessicators and an average and standard deviation of the moisture contents was calculated after equilibration.

TABLE I SORPTION ISOTHERM DATA ($gH_2O/100g$ DRY SOLIDS)

Water Activity	Freeze Dried Batch No. 2 12°C		Freeze Dried Batch No. 2 25°C		Freeze Dried Batch No. 2 40°C	
	Adsorption	Desorption	Adsorption	Desorption	Adsorption	Desorption
0.11	2.74±0.03	3.72±0.01	3.42±0.01	5.53±0.02	1.34±0.01	3.34±0.02
0.23	4.63±0.09	5.18±0.03	5.56±0.03	6.51±0.02	2.15±0.04	4.36±0.02
0.33	--	7.30±0.07	6.19±0.01	7.13±0.06	2.60±0.03	4.43±0.01
0.51	10.66±0.06	9.82±0.02	9.24±0.04	9.71±0.09	4.39±0.01	6.43±0.03
0.68	14.95±0.11	13.86±0.04	15.05±0.10	19.54±0.10	13.11±0.03	15.20±0.09
0.76	18.79±0.09	16.60±0.07	17.99±0.04	23.67±0.01	15.59±0.11	17.35±0.01
0.88	25.67±0.09	24.82±0.11	24.18±0.11	29.85±0.09	28.33±0.08	31.58±0.08
1.00	56.53±0.10	--	64.24 0.51	--	102.06±1.35	--
	Batch No. 3 12°C		Batch No. 3 25°C		Batch No. 3 40°C	
0.11	3.29±0.03	5.39±0.02	5.74±0.01	3.37±0.01	2.89±0.01	4.01±0.01
0.23	6.06±0.01	8.01±0.01	8.25±0.02	6.53±0.01	5.33±0.04	6.39±0.01
0.33	8.76±0.06	10.46±0.05	10.43±0.01	8.81±0.07	6.44±0.05	7.33±0.02
0.55	11.39±0.02	13.31±0.01	12.38±0.03	11.39±0.02	7.37±0.02	8.08±0.01
0.68	13.38±0.01	16.43±0.02	17.17±0.04	16.01±0.04	11.28±0.01	12.01±0.02
0.76	15.56±0.01	17.17±0.01	18.12±0.03	17.15±0.01	12.42±0.02	13.32±0.01
0.88	19.81±0.04	20.36±0.02	23.36±0.08	19.21±0.03	16.69±0.01	16.80±0.01
1.00	34.65±0.01	--	--	32.74±0.06	35.19±0.08	--

Table I cont'd.

Water Activity	Freeze Dried Batch No. 1 25°C		Freeze Dried Batch No. 4 25°C		Freeze Dried Batch No. 5 25°C	
	Adsorption	Desorption	Adsorption	Desorption	Adsorption	Desorption
0.11	3.97±0.01	3.64±0.02	4.56±0.01	6.12±0.03	4.27±0.01	6.60±0.02
0.23	5.21±0.01	7.34±0.03	6.13±0.01	7.96±0.01	6.14±0.01	7.92±0.01
0.33	--	7.81±0.06	8.49±0.01	10.07±0.01	8.67±0.02	10.45±0.01
0.51	9.44±0.03	10.36±0.01	11.90±0.03	11.46±0.01	12.71±0.03	12.86±0.04
0.68	16.63±0.02	14.59±0.02	17.66±0.01	16.39±0	18.27±0.01	18.57±0.01
0.76	25.60±0.07	21.26±0	20.78±0.01	19.86±0.01	20.33±0.01	20.89±0.01
0.88	41.63±0.02	39.82±0.01	22.37±0	22.64±0.02	22.81±0.02	23.66±0.01
1.00	117.10±1.53	--	35.20±0.06	--	31.54±0.01	--
	Drum Dried Batch No. 3 12°C		Drum Dried Batch No. 3 25°C		Drum Dried Batch No. 3 40°C	
0.11	2.54±0.01	3.28±0.02	3.31±0.01	2.81±0.03	2.67±0.01	2.32±0.02
0.23	4.49±0.03	5.15±0.01	5.68±0.03	4.63±0.01	4.25±0.03	3.81±0.03
0.33	6.80±0.01	7.03±0.01	6.84±0.04	6.54±0.01	5.53±0.01	5.29±0.01
0.51	9.20±0.06	10.47±0.02	10.38±0.01	9.18±0.03	5.99±0.01	6.58±0.02
0.68	12.38±0.01	17.52±0.01	17.49±0.01	12.16±0.01	9.97±0.03	10.56±0.03
0.76	15.51±0.01	20.68±0.01	20.38±0.01	15.09±0.01	11.15±0.02	14.10±0.04
0.88	25.30±0.02	29.31±0.03	29.47±0.02	25.38±0.01	23.52±0.01	24.65±0.01
1.00	63.19±0.61	--	61.83±0.84	--	133.91±2.18	--

Table I cont'd.

Water Activity	Spray Dried Batch No. 3 12°C		Spray Dried Batch No. 3 25°C		Spray Dried Batch No. 3 40°C	
	Adsorption	Desorption	Adsorption	Desorption	Adsorption	Desorption
0.11	3.39±0.01	6.29±0.02	3.63±0.03	6.77±0.01	2.88±0.02	4.29±0.03
0.22	6.21±0.09	8.38±0.08	6.41±0.05	8.31±0.01	5.43±0.01	6.79±0.01
0.33	9.00±0.11	11.56±0.09	9.01±0.02	11.08±0.01	7.52±0.03	8.56±0.04
0.51	9.91±0.06	12.27±0.10	10.26±0.01	12.21±0.04	8.85±0.01	9.07±0.03
0.68	13.43±0.04	16.84±0.07	14.92±0.01	16.03±0.01	11.41±0.04	12.20±0.01
0.76	15.35±0.01	17.18±0.04	16.35±0.01	17.85±0	12.57±0.02	13.42±0.03
0.88	19.95±0.01	21.62±0.01	19.34±0.02	22.86±0.01	16.93±0.03	16.73±0.07
1.00	34.81±0.02	--	32.75±0.03	--	31.83±0.01	--
	Whey Protein; Heat Precipitated; Vacuum Dried 25°C		Whey Protein; Heat Precipitated; Air Dried 25°C		Whey Protein; Heat Precipitated; Air Dried 25°C 60 Mesh	
0.11	4.20±0.01	4.21±0.02	1.80±0.03	4.65±0.01	4.16±0.02	4.18±0.01
0.23	5.86±0.02	5.91±0.03	5.14±0.01	7.49±0.03	7.23±0.01	7.31±0.02
0.33	8.39±0.01	8.46±0.01	--	--	10.01±0.01	9.97±0.01
0.51	9.34±0.01	9.87±0.01	8.92±0.01	10.44±0.03	10.39±0.01	11.46±0.01
0.68	14.89±0.02	16.32±0.01	12.87±0.01	14.48±0	14.10±0.02	16.38±0.01
0.76	17.20±0.01	19.98±0	16.53±0.01	17.19±0.01	16.79±0.01	18.96±0.01
0.88	23.98±0.01	25.49±0.01	22.28±0.02	22.68±0.02	23.43±0.01	24.91±0.03
1.00	65.67±0.15	--	59.07±0.04	--	72.50±0.47	--

Table I cont'd.

Water Activity	Whey Protein Curd; Unwashed; 4.135g H_2O/g solids 25°C		Whey Protein Curd; Washed; 3.935g H_2O/g solids 25°C		Whey Protein Curd; Washed; Readsorption 25°C	
	Adsorption	Desorption	Adsorption	Desorption	Adsorption	Desorption
0.11	6.63±0.02	8.61±0.01	0.81±0.01	1.65±0.04	1.73±0.01	2.62±0.01
0.23	7.40±0.01	9.83±0.03	3.82±0.03	6.27±0.03	5.09±0.03	6.06±0.05
0.33	9.36±0.01	11.04±0.03	5.28±0.03	9.99±0.05	6.95±0.05	8.07±0.04
0.51	16.21±0.01	18.33±0.01	6.36±0.04	11.76±0.03	7.46±0.02	9.05±0.03
0.68	17.65±0.03	20.78±0.02	12.31±0.06	--	11.43±0.01	11.71±0
0.76	20.00±0.02	21.37±0.02	--	13.11±0.02	13.35±0.01	13.22±0.01
0.88	26.26±0.01	30.48±0.01	--	16.97±0.01	17.90±0.01	17.11±0.02
1.00	226.38±3.47	396.48±4.16	96.14±0.18	387.19±1.27	63.68±0.27	59.14±0.39
	Freeze Dried Batch No. 2 ; Replicate A 25°C		Freeze Dried Batch No. 2 ; Replicate B 25°C		Freeze Dried Batch No. 3 ; Replicate A 25°C	
0.11	3.49±0.02	5.49±0.04	3.46±0.01	5.57±0.03	3.36±0.02	5.71±0.01
0.23	5.48±0.01	6.54±0.01	5.51±0.02	6.50±0.04	6.49±0.04	8.26±0.03
0.33	6.21±0.03	7.18±0.06	6.15±0.02	7.12±0.03	8.78±0.01	10.39±0.04
0.51	9.19±0.02	9.64±0.03	9.23±0.01	9.68±0.05	11.44±0.07	12.37±0.06
0.68	15.16±0.05	19.63±0.02	15.01±0.04	19.59±0.07	16.07±0.10	17.19±0.13
0.76	17.94±0.04	23.48±0.02	17.87±0.06	23.61±0.09	17.09±0.03	18.07±0.06
0.88	24.31±0.08	29.64±0.07	24.16±0.01	29.73±0.14	19.28±0.06	23.39±0.02
1.00	61.29±1.24	--	66.09±0.09	--	33.56±0.17	--

Table I cont'd.

Water Activity	Freeze Dried Batch No. 3; Replicate B 25°C		Drum Dried Batch No. 3; Replicate A 25°C		Spray Dried Batch No. 3; Replicate A 25°C	
	Adsorption	Desorption	Adsorption	Desorption	Adsorption	Desorption
0.11	3.40±0.05	5.73±0.02	3.37±0.01	2.69±0.03	3.69±0.07	6.71±0.03
0.23	6.55±0.01	8.26±0.01	5.63±0.06	4.61±0.03	6.44±0.01	8.25±0.05
0.33	8.84±0.07	10.37±0.04	6.91±0.03	6.58±0.08	9.07±0.03	10.98±0.04
0.51	11.38±0.12	12.30±0.09	10.37±0.01	9.13±0.02	10.25±0.06	12.27±0.03
0.68	15.98±0.03	17.24±0.0	17.38±0.04	12.24±0.06	14.99±0.01	16.03±0.08
0.76	17.29±0.04	18.01±0.06	20.42±0.04	15.01±0.01	16.38±0.05	17.94±0.01
0.88	19.24±0.01	23.41±0.14	29.41±0.09	25.97±0.08	19.29±0.17	22.87±0.09
1.00	34.07±0.09	--	64.96±1.83	--	33.90±0.14	--
	Spray Dried Batch No. 3; Replicate B 25°		Whey Protein; Dialysed; Denatured 25°C			
0.11	3.64±0.03	6.74±0.02	7.68±0.01	8.28±0.02		
0.23	6.39±0.01	8.29±0.04	10.04±0.02	13.92±0.02		
0.33	8.99±0.05	11.01±0.01	--	--		
0.51	10.19±0.07	12.26±0.06	16.84±0.01	17.15±0.01		
0.68	14.87±0.06	15.99±0.01	19.58±0.01	21.12±0.02		
0.76	16.39±0.09	17.90±0.03	21.68±0.03	23.90±0.01		
0.88	19.27±0.03	22.91±0.10	25.40±0.01	26.88±0.01		
1.00	34.07±0.12	--	40.02±0.07	--		

Table I continued

Lactose Glass 25°C		Whey Protein; Dialysed Undenatured 25°C	
Adsorption	Desorption	Adsorption	Desorption
0.55±0.01	4.43±0.08	6.09±0.02	7.78±0.01
0.69±0.03	5.01±0.03	--	9.34±0.02
0.79±0.02	4.45±0.03	11.76±0.03	11.44±0.01
0.73±0.06	3.81±0.01	12.80±0.01	13.85±0.01
1.52±0.03	5.39±0.02	16.29±0.04	17.82±0.04
1.88±0.04	4.33±0.04	19.04±0.01	18.64±0.01
2.33±0.01	4.88±0.01	26.54±0.01	27.56±0.03
40.61±0.15	--	70.64±0.12	--
Freeze Dried Acid Whey 25°C		**Whey Protein; Alkali Solubilised 25°C**	
3.67±0.01	5.17±0.02	4.38±0.02	4.91±0.03
6.33±0.02	5.99±0.01	8.60±0.04	9.12±0.02
7.55±0.01	8.49±0.01	13.38±0.01	13.48±0.03
7.87±0.02	15.33±0.02	18.01±0.03	17.79±0.01
17.31±0.01	18.30±0.01	28.88±0.02	24.43±0.04
23.89±0.01	21.51±0	36.32±0.01	30.66±0.01
39.61±0.02	35.82±0.02	46 01±0.06	48.07±0.02
116.11±1.65	--	253.99±2.61	--

APPENDIX B

KNOT POSITIONS AND COEFFICIENT MATRICES FOR CURVE FITTING

Using the experimentally obtained sorption isotherm data, a series of adsorption/desorption isotherms were constructed. To obtain a curve a coefficient matrix (C) is used to construct a series of points (X) about which the curve pivots. These points are known as knots or knot positions. After the knot positions have been calculated, it is then possible to construct a series of points through which a curve is drawn. The following equation is used to calculate the points on the curve:-

$$X = X(I) + D\,[C(1,I) + D.C(2,I) + D.C(3,I)]$$

where $D = a_w - a_w(I)$

and X = calculated moisture content

$X(I)$ = value of moisture content at knot I

$C(1,I)$, $C(2,I)$, $C(3,I)$ — I^{th} row of the coefficient matrix C

As an example, assume the knots have been placed at 0.0, 0.20, 0.30 and 0.80. To calculate the moisture content at $a_w = 0.05$ we assume that $0 < a_w < 0.2$ i.e. $a_w(I) = a_w(1) = 0$

$\therefore$ $D = 0.05 - 0.00 = 0.05$

if $I = i$, then $X(i) = -2.46998$

and $C(1,1) = 105.214$

$C(2,1) = -569.445$

$C(3,1) = 1125.30$

Using the above, it is possible to construct a series of calculated moisture contents at each of the data points. When completed, a curve is constructed. The following table shows the knot positions used to

construct the curves, the water content values [X(I)] at each of these knots and the coefficient matrices used to fit the sorption isotherms.

TABLE II KNOT POSITIONS, WATER CONTENT VALUES AND COEFFICIENT MATRICES USED TO FIT SORPTION ISOTHERMS

Description	Knot Position $[a_w(I)]$	Water Content X(I)	C_1	C_2	C_3
Freeze Dried Batch No. 2; 12°C Adsorption	0.00	0.1345E-02	35.7922	-128.887	265.885
	0.20	4.1314	16.1438	30.6436	-50.4226
	0.45	9.2947	22.0114	-7.1743	102.263
	0.80	23.4962	65.3583	115.549	6112.38
Freeze Dried Batch No. 2; 12°C Desorption	0.00	-2.4699	105.214	-569.445	1125.30
	0.20	4.7975	12.4724	105.735	-474.354
	0.30	6.6277	19.3888	-36.5715	91.1377
	0.80	18.5715	51.1706	100.137	2961.07
Freeze Dried Batch No. 2; 12°C Adsorption	0.00	0.3203E-04	35.2513	-28.1811	-87.6679
	0.20	5.2217	13.4588	-80.7794	265.741
	0.42	7.1025	16.5014	94.6112	-156.371
	0.65	14.0002	32.2065	-13.2876	208.803
	0.85	22.1804	54.9478	112.002	9273.31
Freeze Dried Batch No. 2; 25°C Desorption	0.00	-16.3116	584.946	-5326.98	16494.8
	0.1069	5.4949	11.5229	-36.2361	78.1781
	0.4000	7.7276	10.4284	32.5024	332.089
	0.6000	13.7701	63.2802	231.745	-1514.99
	0.7384	22.9503	40.3803	-397.224	3220.99
Freeze Dried Batch No. 2; 40°C Adsorption	0.000	0.6521E-04	15.7851	-37.1617	39.9092
	0.290	2.4258	4.3004	-2.4349	90.2839
	0.420	3.1420	8.2447	32.7841	330.039
	0.650	10.7882	75.7025	260.226	-6816.08

Table II cont'd.

Description	Knot Position $[a_w(I)]$	Water Content X(I)	C_1	C_2	C_3
Freeze Dried	0.0000	-4.1782	129.322	-702.485	1347.09
Batch No. 2; 40°C	0.1584	4.0342	8.1753	-62.4544	164.491
Desorption	0.4000	4.6865	6.8395	56.9288	227.129
	0.6132	10.9344	62.0921	202.211	-2628.33
	0.7044	16.2850	33.4042	-516.785	4684.64
Whey Protein	0.0000	-0.2676E-04	-0.7524E-01	187.012	-394.112
Curd; Washed	0.2000	4.3125	27.4362	-49.4558	-42.5287
Readsorption;	0.4000	7.4813	2.5505	-74.9736	454.729
25°C	0.5967	8.5420	25.8238	193.308	-1048.13
	0.7592	13.3454	5.6182	-317.659	4825.43
Whey Protein	0.0000	-1.7692	47.0418	-72.6812	70.5467
Curd	0.2000	5.2963	26.4349	-30.3571	-68.3483
(Readsorption)	0.4025	8.8372	5.7279	-71.8831	344.525
25°C	0.5984	9.7906	17.2267	130.580	-654.939
Desorption	0.7724	13.2913	3.1778	-211.313	4757.39
Whey Protein	0.0000	0.5718E-04	-5.3191	120.980	-51.6193
Curd; Washed;	0.1131	0.8718	20.0723	130.468	-507.969
Adsorption	0.2508	4.2713	19.6727	-106.366	237.512
25°C	0.5879	7.9146	28.9335	133.840	765.771
Whey Protein	0.00	0.3573	-1.4498	215.377	-355.387
Curd; Washed;	0.31	9.3037	29.6257	-115.134	150.753
Desorption;	0.85	15.4667	37.1601	129.084	11361.3
25°C					

Table II cont'd.

Description	Knot Position $[a_w(I)]$	Water Content X(I)	C_1	C_2	C_3
Drum Dried	0.0000	-0.8265E-01	41.6789	-101.672	118.194
Batch No. 3	0.5626	12.2317	39.5081	97.8089	-466.814
Adsorption; 25°C	0.7208	19.0802	35.4147	-123.693	1952.15
Drum Dried	0.0000	-6.7296	341.786	-4430.02	19819.7
Batch No. 3	0.0750	2.3468	11.7392	29.3246	-46.5110
Desorption; 25°C	0.4600	8.5588	13.6369	-24.3957	169.702
	0.770	15.4974	47.4366	133.426	2303.80
Drum Dried	0.0000	-.4428E-04	47.2226	-320.646	920.628
Batch No. 3	0.1500	2.9759	13.1713	93.6328	-271.150
Adsorption; 12°C	0.3432	7.0597	18.997	-63.5037	159.69
	0.8014	17.8212	61.5691	156.422	3444.96
Drum Dried	0.0000	-1.5498	89.3413	-570.455	1431.06
Batch No. 3	0.140	3.7050	13.7653	30.9197	-59.0459
Desorption; 12°C	0.4017	8.3655	17.8192	-15.4242	280.081
	0.5999	13.4724	44.7154	151.105	-977.872
	0.7212	19.3750	38.2035	-204.792	2256.82
Freeze Dried	0.0000	0.1270E-04	82.3222	-550.200	1406.44
Batch No. 4	0.1500	4.7156	12.1971	82.6985	-157.807
Adsorption; 25°C	0.4300	11.1502	21.3920	-49.8624	327.145
	0.6431	16.6087	44.6976	159.302	-4107.53
	0.6760	18.1069	41.8237	-246.499	757.333
Freeze Dried	0.0000	-4.1540	188.622	1040.59	1951.34
Batch No. 4	0.2172	7.7187	12.7588	230.906	-1145.68
Desorption; 25°C	0.3180	10.1775	24.3871	-115.548	318.537
	0.6400	16.6844	49.0556	192.143	-6305.65
	0.6625	17.8136	48.1253	-233.504	625.287

Table II cont'd.

Description	Knot Position $[a_w(I)]$	Water Content X(I)	C_1	C_2	C_3
Whey Protein Vacuum Dried Adsorption; 25°C	0.0000	-0.8041E-04	93.9486	-719.705	1933.99
	0.1500	4.4261	8.5813	150.580	-421.800
	0.3900	9.3280	7.9728	-153.113	729.401
	0.5900	10.6333	34.2556	284.533	-1553.23
	0.7288	16.7164	23.4627	-362.282	3471.39
Whey Protein Vacuum Dried Desorption; 25°C	0.0000	-0.5316	112.737	-913.693	2551.62
	0.1392	4.3394	6.6916	151.897	-386.641
	0.3900	9.4726	9.9248	-138.996	698.973
	0.5912	11.5375	38.9048	283.000	-1286.51
	0.7579	19.9273	26.0123	-360.333	4264.68
Spray Dried Batch No. 3 Adsorption 25°C	0.0000	0.1144	29.9215	7.8071	-55.2706
	0.4429	10.0966	4.3006	-65.6421	634.082
	0.6238	12.4752	42.7371	278.631	-4946.60
	0.6633	14.2958	41.5559	-308.117	1032.21
Spray Dried Batch No. 3 Desorption; 25°C	0.0000	-7.5042	237.744	-1207.98	2057.69
	0.2500	8.5843	19.5696	335.369	-2376.51
	0.3400	11.3288	22.1690	-306.395	1256.05
	0.4600	11.7474	2.8956	145.803	-328.391
	0.6400	15.0774	23.4650	-31.5314	284-559

Table II cont'd.

Description	Knot Position $[a_w(I)]$	Water Content X(I)	C_1	C_2	C_3
Freeze Dried	0.0000	0.1864E-04	93.4566	-656.539	1670.83
Batch No. 4	0.1497	4.8825	9.2152	93.7213	-187.881
Adsorption 25°C	0.4300	10.6918	17.4681	-64.2808	433.882
	0.6400	15.5435	47.8728	209.068	-2077.40
	0.7335	20.1506	32.4543	-373.894	1746.33
Freeze Dried	0.0000	-3.6599	196.028	-1316.22	3112.73
Batch No. 4	0.1500	6.6347	11.2700	84.4985	-228.276
Desorption 25°C	0.4300	11.4039	4.8968	-107.247	685.212
	0.6400	14.0487	50.5084	324.426	-3103.85
	0.7139	18.3008	47.6008	-363.758	1411.58
Freeze Dried	0.0000	0.1868E-01	30.9196	-3.7191	-28.7367
Batch No. 3	0.4000	9.9523	14.1507	-38.2026	259.660
Adsorption 25°C	0.6067	13.5369	31.6293	122.768	-1278.33
	0.7021	16.5624	20.1337	-243.238	1201.49
Freeze Dried	0.0000	-25.9416	1014.73	-11592.2	45884.5
Batch No. 3	0.0500	1.5500	199.646	-4709.53	45837.3
Desorption 25°C	0.0836	4.6785	38.4182	-94.6057	112.174
	0.5599	13.6387	24.6580	65.7144	-454.213
	0.6486	16.0238	25.6042	-55.0472	335.627
Spray Dried	0.0000	0.2923E-04	53.9308	-305.608	862.023
Batch No. 3	0.1500	4.1326	20.8440	85.0296	-282.963
Adsorption 12°C	0.4400	10.4372	-1.2306	-161.147	1014.80
	0.6200	10.9135	39.3954	386.870	-6393.07
	0.6532	12.4121	43.9628	-249.112	889.598
Spray Dried	0.00	-9.2117	325.274	-2276.05	5445.56
Batch No. 3	0.15	6.7472	10.0361	174.464	-463.314
Desorption 12°C	0.43	13.0646	-1.2356	-214.710	1446.49
	0.58	12.9302	31.9895	436.325	-4647.57
	0.63	15.0395	40.7653	-260.692	798.690

Table II cont'd.

Description	Knot Position $[a_w(I)]$	Water Content X(I)	C_1	C_2	C_3
Freeze Dried	0.0000	-22.02E-04	72.2687	-448.261	1085.24
Batch No. 1	0.1344	4.2507	10.5850	-10.6316	51.2182
Adsorption 25°C	0.6012	12.0851	34.1418	61.1452	3197.24
	0.6547	14.5712	68.0533	573.648	-2144.45
	0.7945	29.4377	102.709	-325.751	9248.30
Freeze Dried	0.0000	-13.7104	253.573	-1024.12	1389.38
Batch No. 1	0.2567	7.3991	2.4463	45.7252	-36.4904
Desorption 25°C	0.6030	12.2140	20.9878	7.7897	1558.45
	0.7078	16.2955	74.0054	497.897	-1802.14
	0.8000	25.9399	119.859	- .6170	8389.14
Freeze Dried	0.0000	- .6930E-05	43.3191	-165.998	400.840
Batch No. 3	0.2240	5.8796	29.2895	103.359	-1658.72
Adsorption 12°C	0.2600	6.9905	30.2822	-75.7902	97.4002
	0.9280	22.4317	59.4102	119.405	1960.27
Freeze Dried	0.0000	-4.2993	163.317	-876.605	1751.54
Batch No. 3	0.1857	7.0151	18.9406	98.9654	-454.116
Desorption 12°C	0.3004	9.8046	23.7188	-57.3112	114.163
	0.5997	14.8315	20.0968	45.1979	-591.442
	0.6962	16.6606	12.2906	-126.080	918.370
Spray Dried	0.0000	0.5249E-01	24.4034	9.0801	-46.0005
Batch No. 4	0.4481	3.6721	4.8279	-52.7594	403.041
Adsorption 40°C	0.6112	9.8040	19.7720	144.408	-1403.23
	0.6769	11.3291	20.5680	-132.302	802.502

Table II cont'd.

Description	Knot Position $[a_w(I)]$	Water Content X(I)	C_1	C_2	C_3
Spray Dried Batch No. 3 Desorption 40°C	0.0000 0.1500 0.4005 0.5992 0.6592	-4.5632 5.1636 9.1781 9.9045 12.5626	159.578 19.3253 3.2517 22.1021 21.1929	-959.645 24.6196 -88.7754 183.656 -193.130	2187.37 -150.868 457.162 -1307.79 1085.03
Drum Dried Batch No. 3 Desorption 40°C	0.0000 0.2088 0.2520 0.8560	-0.4996 3.4139 4.2327 21.7648	47.2202 16.6810 18.4622 95.7024	-262.896 116.679 -75.4086 203.283	605.895 -1482.45 153.807 31225.5
Drum Dried Batch No. 3 Adsorption 40°C	0.0000 0.3789 0.60000 0.7625	0.6338E-01 5.6251 7.4441 11.2159	25.6697 3.0381 25.7752 -3.9124	-27.3084 -32.4109 135.274 -317.947	-4.4883 252.882 -929.538 10569.9
Freeze Dried Batch No. 3 Adsorption 40°C	0.0000 0.2938 0.6071 0.6988	0.684E-03 6.1514 9.1042 11.6665	27.6333 9.4252 26.8242 19.9792	-6.4362 -55.5351 111.077 -185.717	-55.7013 177.282 -1078.65 1257.54
Freeze Dried Batch No. 3 Desorption 40°C	0.0000 0.1500 0.4419 0.6000 0.7074	-2.6524 5.0211 7.7972 9.4745 12.6911	101.751 21.4333 2.8934 26.7466 19.6637	-476.432 -59.0196 -4.5027 155.344 -221.284	927.588 62.2629 336.957 -1168.84 1421.21

Table II cont'd.

Description	Knot Position $[a_w(I)]$	Water Content X(I)	C_1	C_2	C_3
Freeze Dried	0.0000	-4.8099	175.761	-968.180	1774.46
Acid Whey;	0.2175	5.8746	6.4217	189.517	-346.160
Desorption;	0.5284	15.7884	23.8707	-133.403	584.812
25°C	0.8117	25.1421	89.1003	363.639	9171.32
Whey Protein	0.0000	-0.1015E-04	130.981	-724.612	1532.65
Denatured	0.1670	8.8035	17.1934	43.3014	-73.2282
Adsorption;	0.6003	18.4255	13.4766	-51.8603	808.877
25°C	0.6748	19.4768	19.2270	129.030	-600.384
	0.7882	22.4418	25.3237	-75.2811	1642.08
Whey Protein	0.0000	-2.9717	137.085	-352.654	330.205
Denatured	0.2783	14.9831	17.5208	-76.9632	180.009
Desorption;	0.6176	19.0991	27.4645	106.270	-438.002
25°C	0.8001	24.9894	22.4776	-133.588	1860.89
Whey Protein	0.00	-0.2189E-02	67.4890	-108.632	15.6515
Undenatured	0.15	7.7298	35.9559	-101.590	119.561
Adsorption; 25°C	0.85	24.1292	69.4847	149.500	9695.76
Whey Protein	0.00	-7.1822	287.063	-1826.23	4065.96
Undenatured	0.15	8.5097	13.6469	3.4521	4.7867
Desorption; 25°C	0.65	16.7946	20.6891	10.6308	-160.173
	0.75	18.8096	18.0101	-37.4196	3177.20
Lactose Glass	0.00	0.1734	3.0877	-6.2110	6.2530
Adsorption; 25°C	0.86	2.2125	6.2790	9.9141	13601.8
Lactose Glass	0.00	-4.3241	123.185	-485.132	576.613
Desorption; 25°C	0.31	4.4198	-11.3598	51.114	-29.4915
	0.51	3.9565	5.5468	33.4187	-172.533
	0.80	4.1676	-18.6004	-116.682	5603.73

Table II cont'd.

Description	Knot Position $[a_w(I)]$	Water Content X(I)	C_1	C_2	C_3
Whey Protein Curd; Unwashed Adsorption; 25°C	0.000	-0.5431E-04	-29.4141	2205.15	-13248.2
	0.072	4.3687	82.0906	-626.34	1633.21
	0.272	7.5989	15.3930	323.578	1053.32
	0.430	13.9772	38.9048	-175.711	333.747
	0.880	26.3158	83.5168	274.841	107688
Whey Protein Curd; Washed Desorption; 25°C	0.0000	-9.2075	305.822	-1618.74	2827.81
	0.1997	9.8301	-2.3799	75.7157	106.206
	0.3959	13.0767	39.5736	138.194	-715.412
	0.5808	20.5976	17.2578	-258.833	1041.50
	0.9040	34.2923	176.262	750.886	170340
Whey Protein; Alkali Solubilised Adsorption; 25°C	0.00	0.3213E-02	52.6707	-168.000	449.597
	0.20	7.4141	39.4223	101.755	-440.662
	0.40	15.8435	27.2449	-162.648	914.305
	0.57	20.2666	51.2147	303.651	-652.291
	0.87	45.3477	57.2866	-283.393	93757.1
Whey Protein; Alkali Solubilised Desorption; 25°C	0.00	0.6465	68.7202	-221.227	476.443
	0.20	8.0599	37.4024	64.6314	-301.674
	0.40	15.7123	27.0541	-116.372	467.982
	0.57	19.2476	28.0617	122.301	277.127
	0.87	46.1556	176.267	371.697	80859.4
Freeze Dried Acid Whey Adsorption; 25°C	0.0000	0.1527E-01	37.114	-34.8559	-26.4988
	0.3766	7.6335	-0.4174	-64.7953	615.345
	0.5824	10.1656	51.0841	315.072	-938.203
	0.7678	24.4861	71.1751	-206.691	6886.69

Table II cont'd.

Description	Knot Position $[a_w(I)]$	Water Content X(I)	C_1	C_2	C_3
α Lactose (Hydrate) Adsorption; 25°C	0.000 0.072	-0.1013E-05 2.1941	67.2843 10.8914	-750.560 -32.6762	3323.54 27.3956
α Lactose (Hydrate) Desorption; 25°C	0.00 0.09	-0.3633 2.4212	74.7189 7.9374	-717.296 -24.7207	2565.10 21.8626
Whey Protein Air Dried; 60# Adsorption; 25°C	0.00 0.15 0.39 0.59 0.78	0.3357E-03 5.0628 10.7632 10.9835 17.4724	63.2987 24.1817 6.2337 19.6750 22.9403	-330.20 69.4102 -144.191 211.402 -194.218	888.044 -296.672 592.647 -711.610 5577.18
Whey Protein Air Dried, 60# Desorption; 25°C	0.0000 0.1198 0.3900 0.5912 0.7584	-1.2563 4.3805 10.9533 12.7684 18.9356	108.177 21.0685 10.2855 29.8138 15.4470	-803.418 76.0149 -115.909 212.966 298.899	2447.90 -236.737 544.818 -1020.68 4734.32
Whey Protein Air Dried Adsorption; 25°C	0.000 0.20 0.62 0.66 0.80	0.8929E-05 4.4137 10.9497 12.1081 18.1277	-0.9392 26.2926 24.6741 34.7780 33.7226	208.953 -72.7940 68.9369 183.654 -191.188	-469.579 112.488 956.029 -892.491 5230.67
Whey Protein Air Dried Desorption; 25°C	0.00 0.20 0.62 0.66 0.80	-5.9112 7.0765 12.6714 13.8358 18.4417	157.207 14.8842 26.6588 31.3266 29.1209	-672.415 -39.1992 67.2561 49.4518 -65.2094	1055.36 84.4709 -148.477 -272.998 4542.00

APPENDIX C

BET MONOLAYER CALCULATIONS

After constructing the isotherms using the method outlined in Appendix B, an analysis was made of the isotherm between a_w 0.10 and 0.35 to determine the BET monolayer moisture content. The computer program used in Appendix B was modified to print out a series of data points on the curve between a_w 0.10 and 0.35.

The BET equation is of the form:-

$$\frac{a}{(1-a)m} = \frac{1}{m_o c} + \frac{(c-1)a}{m_o c} \qquad [1]$$

where a = water activity of a given sample at a given moisture content

m = given moisture content of sample; g water/g dry solids

m_o = monolayer moisture content; g water/g dry solids

c = energy constant

Using the Salwin rearrangement of the above equation we have:-

$$\frac{a}{m(1-a)} = I + s.a \qquad [2]$$

where $m_o = \frac{1}{I+S}$

where I = intercept

and S = slope of a plot of $\frac{a}{m(1-a)}$ against a

Table III is an example of the monolayer raw data. The raw data for each isotherm were plotted and I and S were determined. The BET thermodynamic constant (c) was calculated from the value of the intercept (I) where:-

$$I = \frac{1}{m_o c} \qquad [3]$$

TABLE III - BET MONOLAYER DATA EXAMPLE

Water Activity	Batch No. 3; 25°C Spray Dried (Repeat) B	
	m	$\frac{a}{m(1-a)}$
0.10	0.0321	3.4602
0.11	0.0351	3.5256
0.13	0.0402	3.7249
0.15	0.0466	3.7879
0.17	0.0522	3.9261
0.19	0.0577	4.0685
0.21	0.0629	4.2254
0.23	0.0679	4.3977
0.25	0.0727	4.5872
0.27	0.0772	4.7872
0.29	0.0813	5.0259
0.31	0.0852	5.2721
0.33	0.0887	5.5556
0.35	0.0915	5.8824

APPENDIX D

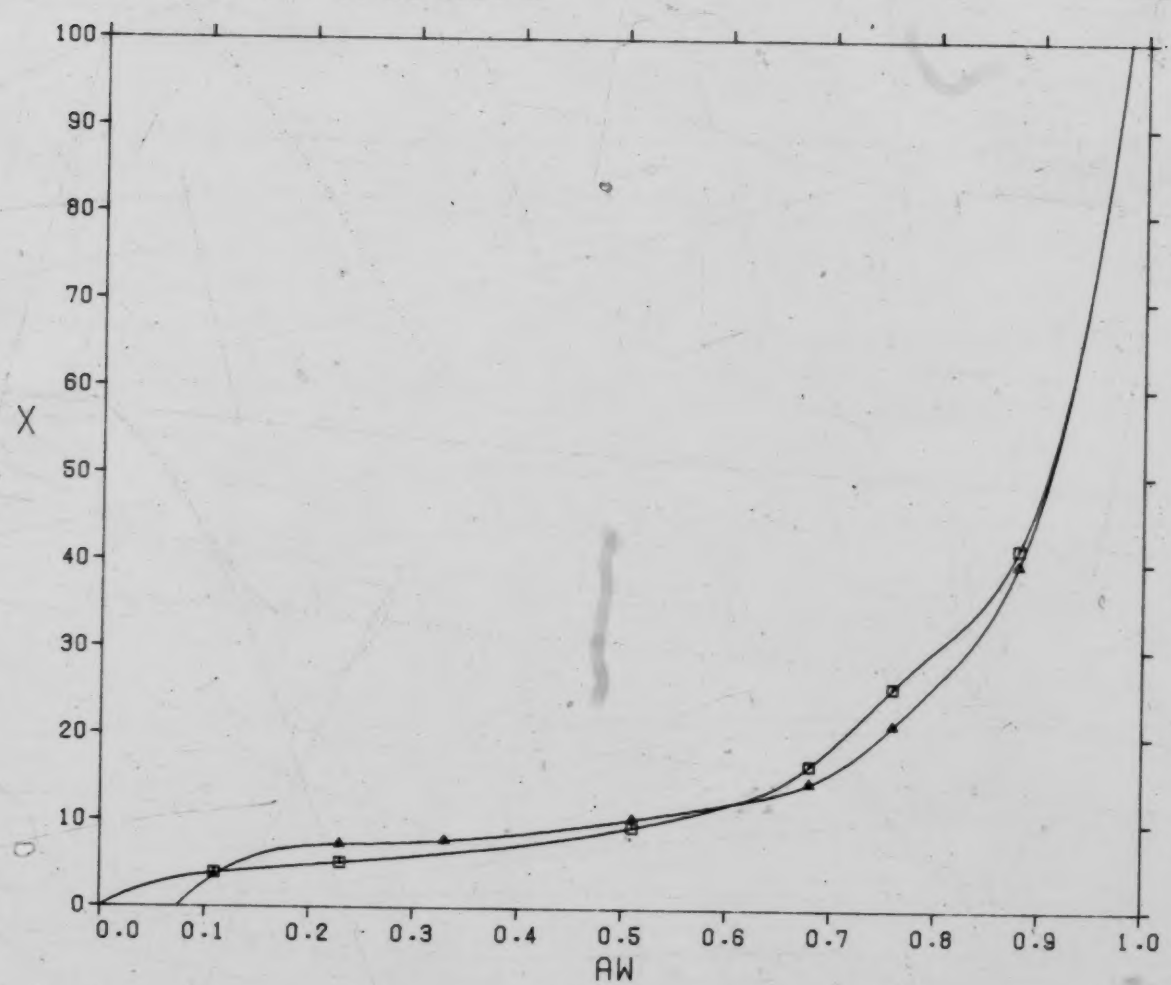
ISOTHERMS AND PORE DISTRIBUTION CURVES

Moisture sorption isotherms and pore distribution curves for whey protein powders and components.

WHEY PROTEIN

HEAT-PRECIPITATED, FREEZE-DRIED, BATCH NO. 1
UNWASHED

TEMPERATURE 25.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

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WHEY PROTEIN

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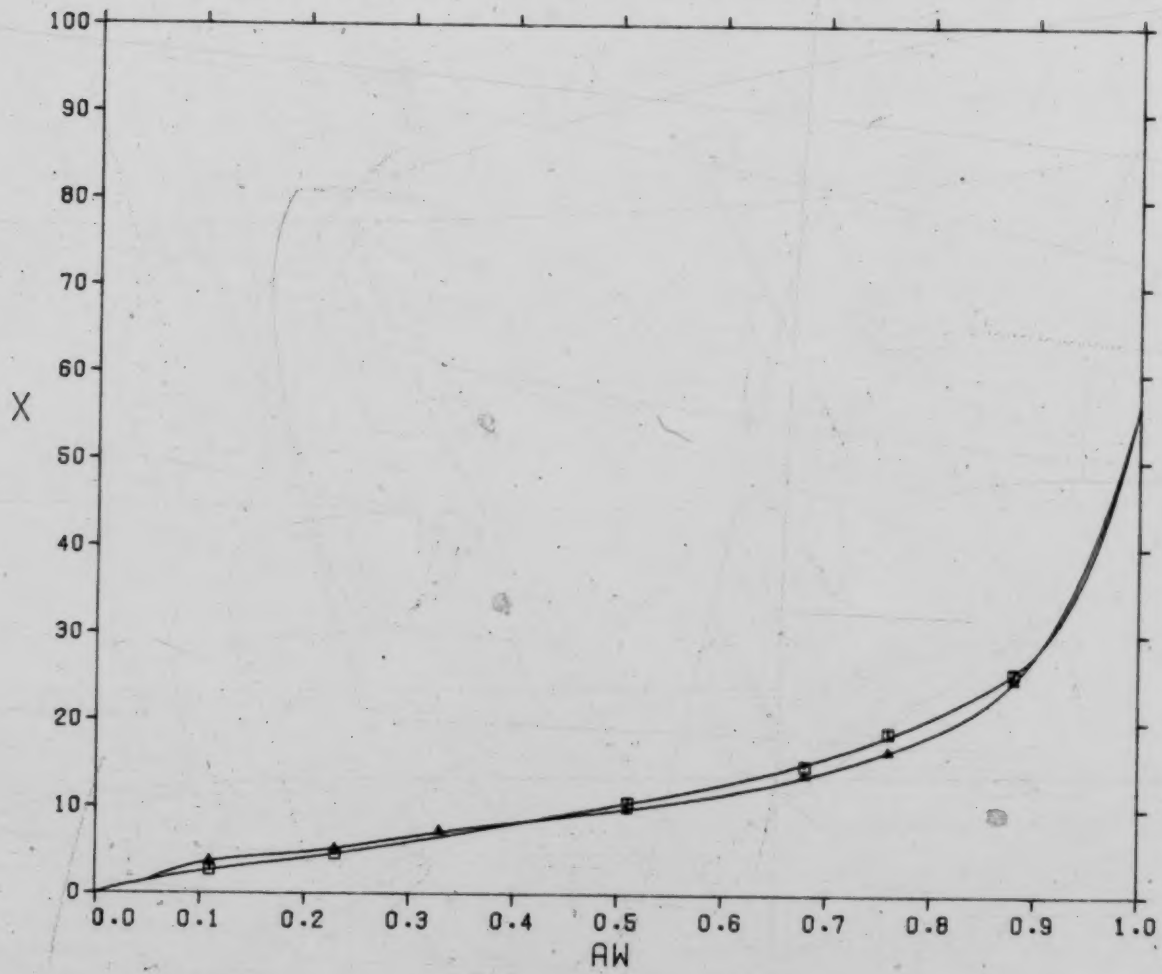
UNWASHED

TEMPERATURE 12.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

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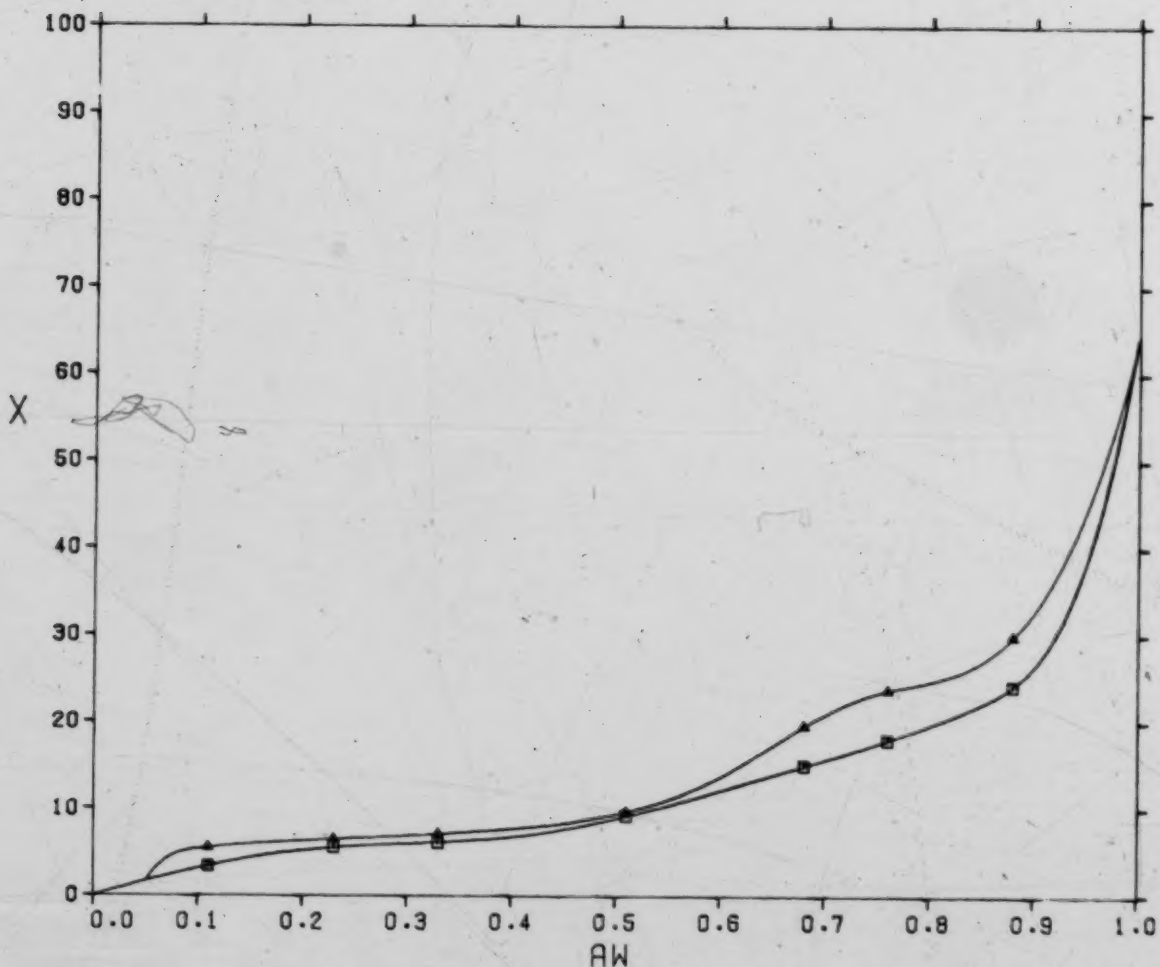
WHEY PROTEIN

HEAT-PRECIPITATED, FREEZE-DRIED; BATCH NO. 2
UNWASHED

TEMPERATURE 25.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

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WHEY PROTEIN

HEAT-PRECIPITATED, FREEZE-DRIED, BATCH NO. 2
UNWASHED

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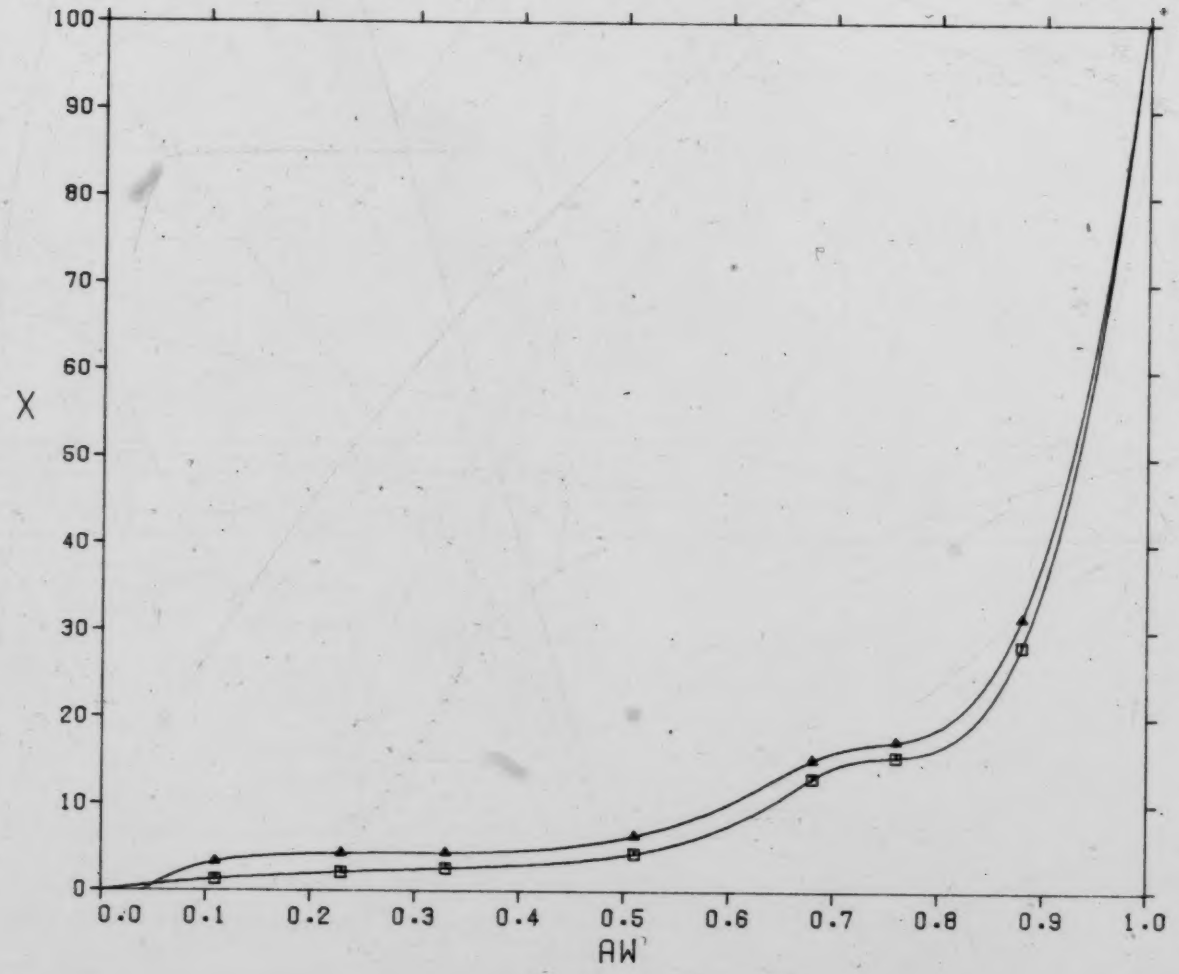

X (KG WATER/100 KG DRY MATTER)

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WHEY PROTEIN

HEAT-PRECIPITATED, SPRAY-DRIED
WASHED, BATCH NO. 3

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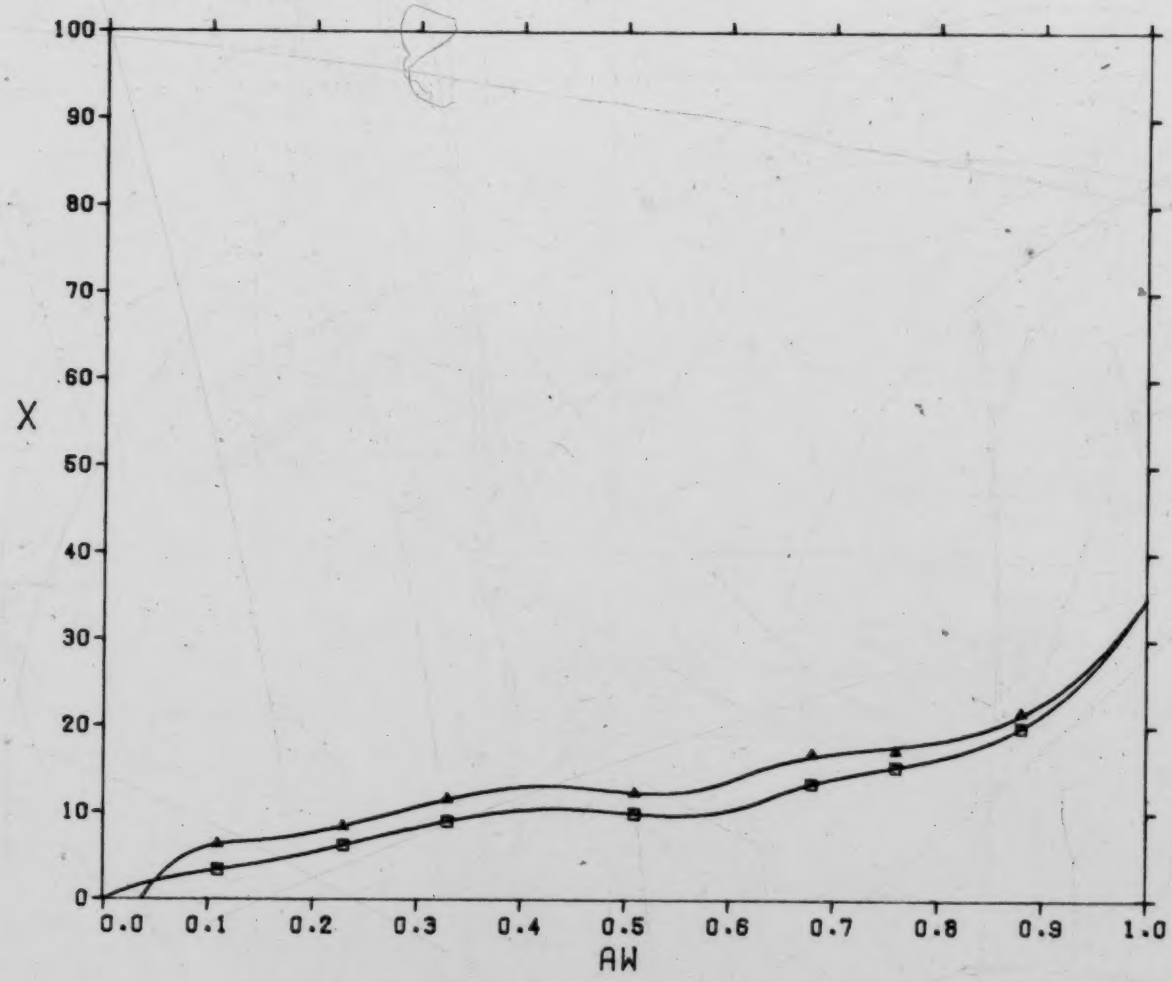

X (KG WATER/100 KG DRY MATTER)

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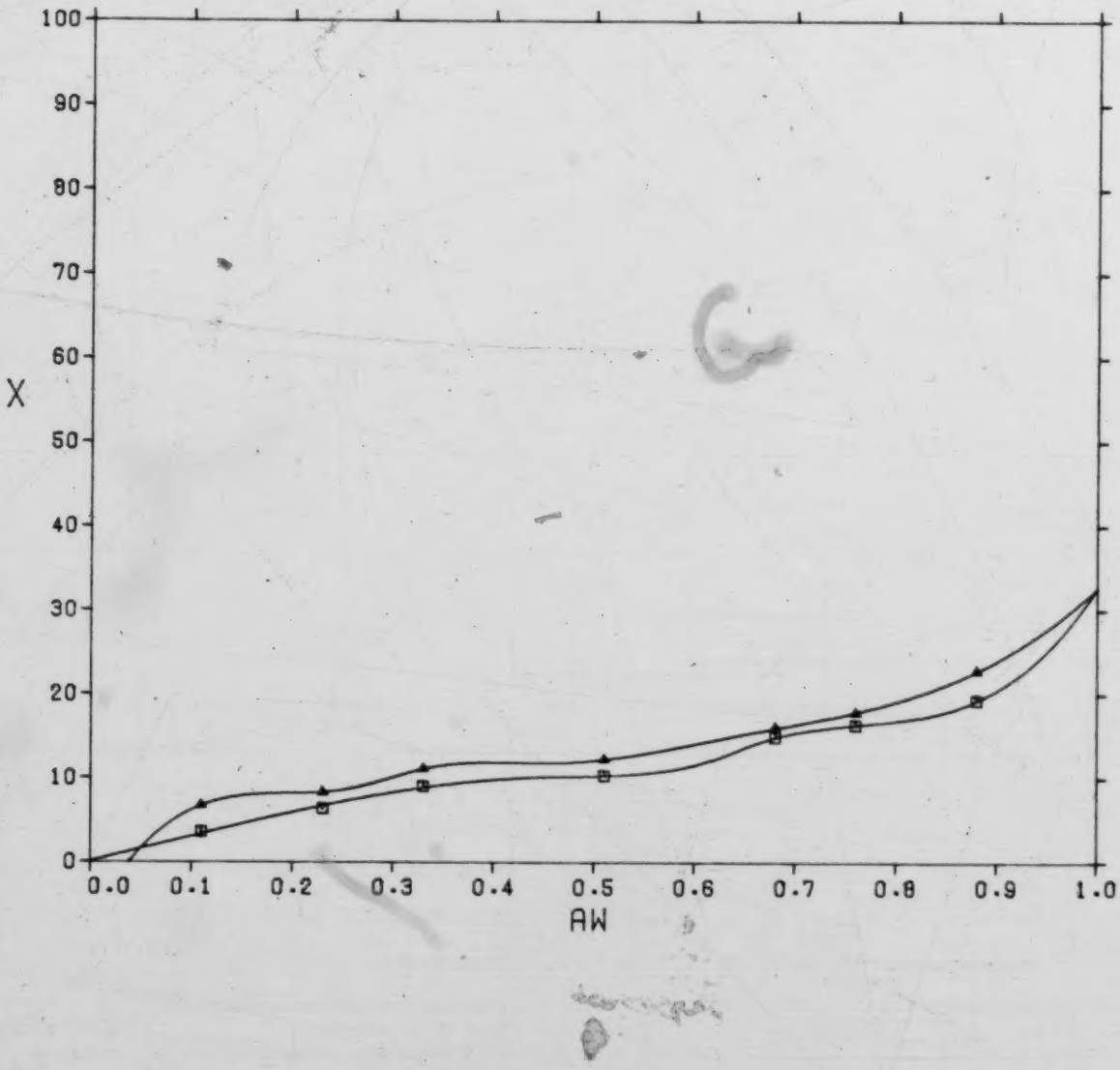
WHEY PROTEIN

HEAT PRECIPITATED SPRAY DRIED
WASHED, BATCH NO. 3

TEMPERATURE 25.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

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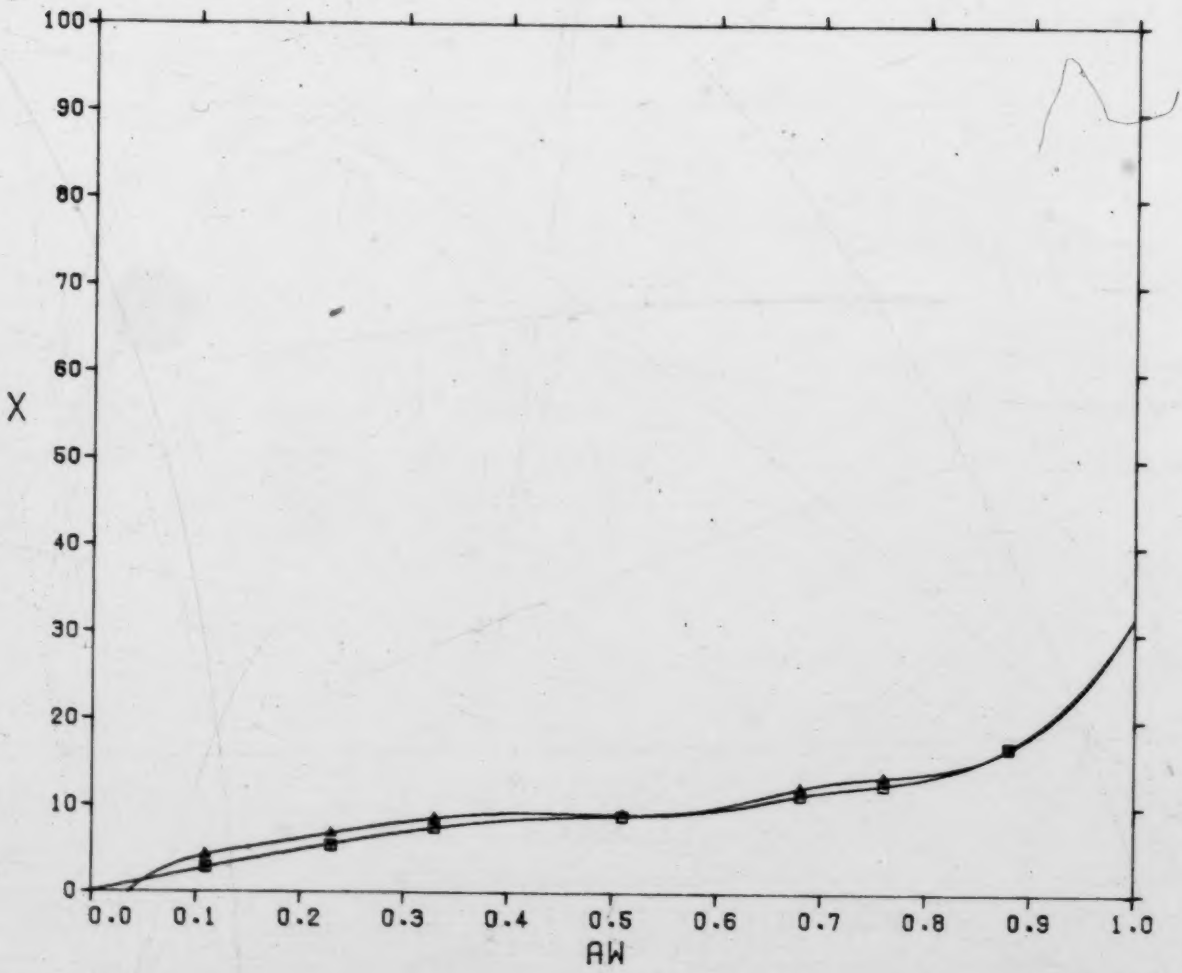
WHEY PROTEIN

HEAT PRECIPITATED SPRAY DRIED
BATCH NO. 3

TEMPERATURE 40.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

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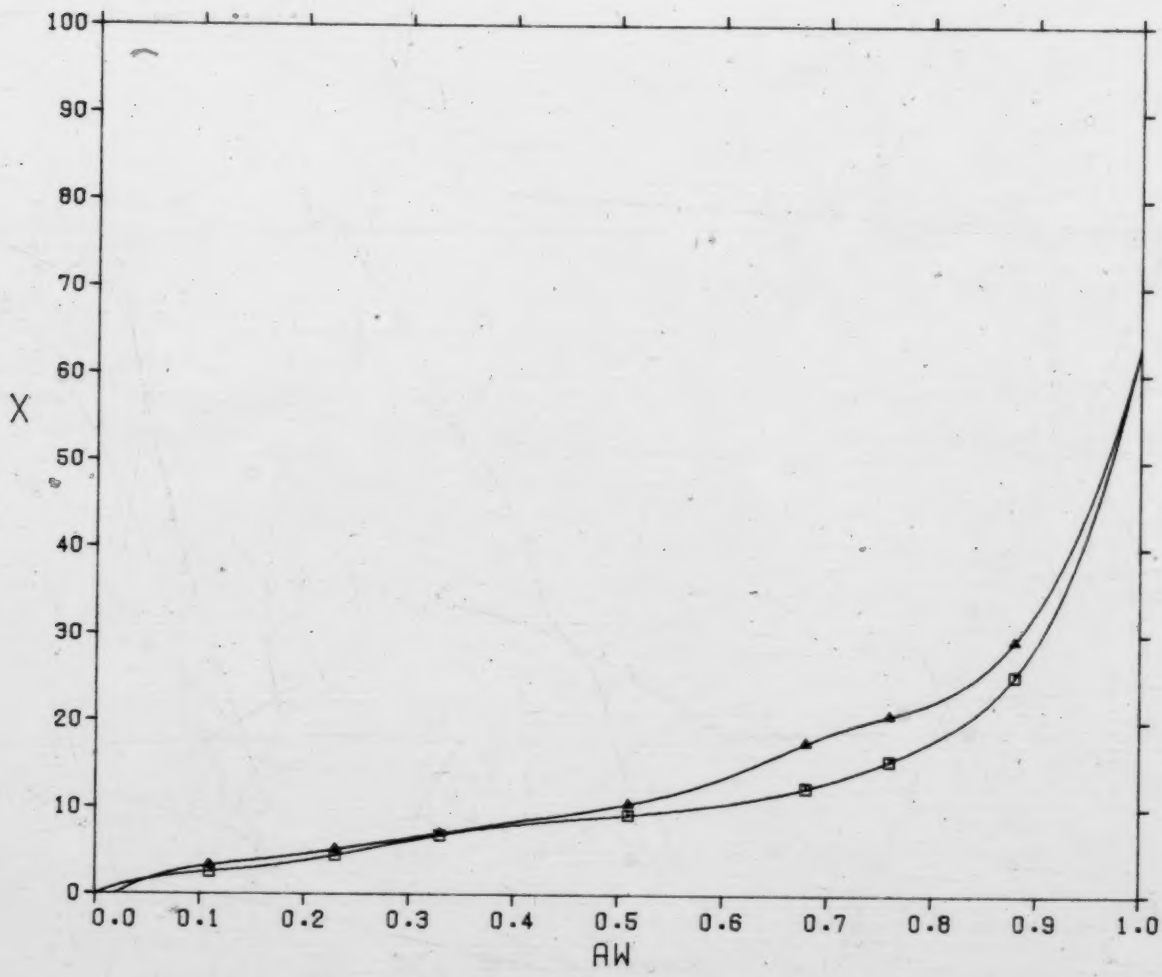
WHEY PROTEIN

HEAT-PRECIPITATED, DRUM-DRIED
WASHED, BATCH NO. 3

TEMPERATURE 12.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

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WHEY PROTEIN

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WASHED, BATCH NO. 3

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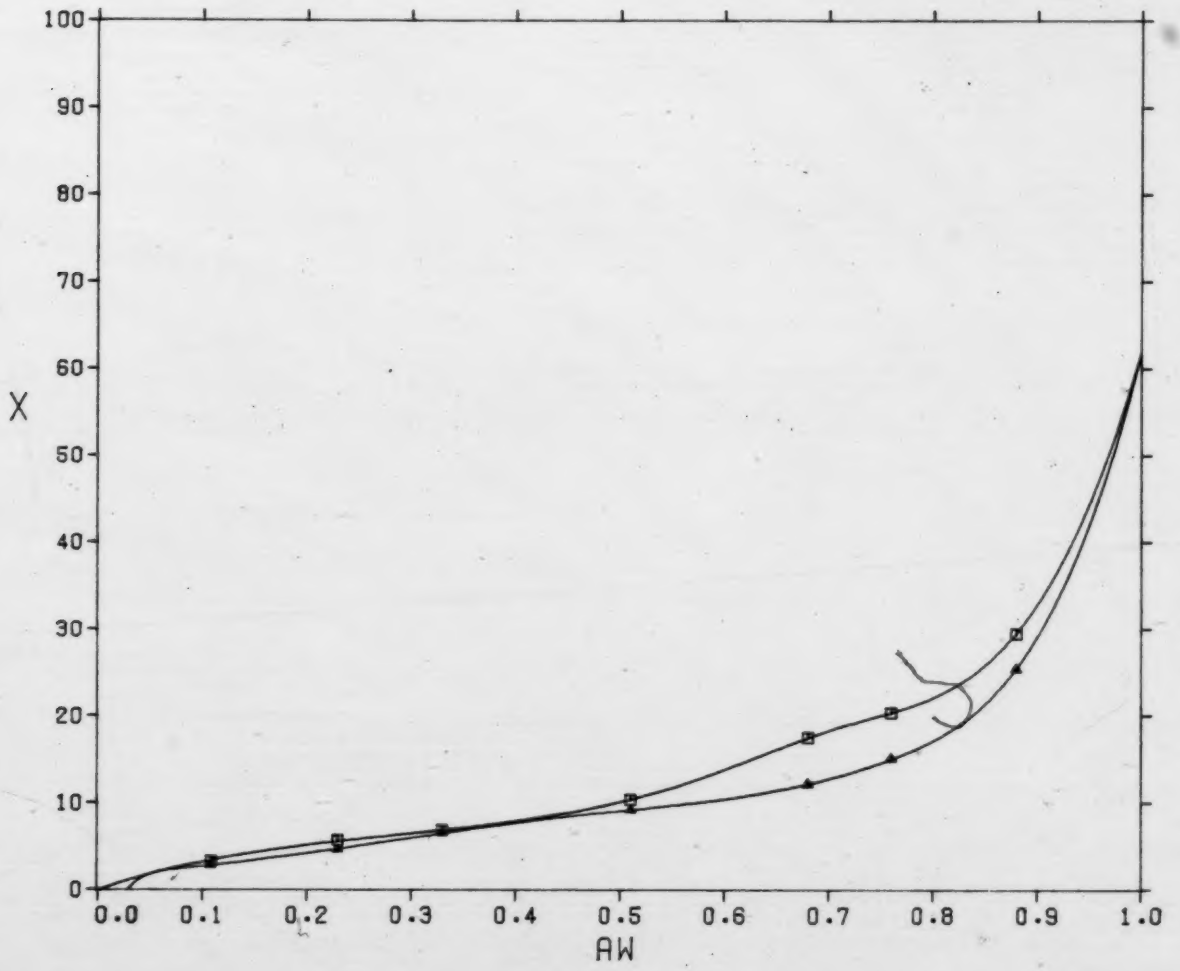

X (KG WATER/100 KG DRY MATTER)

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WHEY PROTEIN

HEAT PRECIPITATED DRUM DRIED
BATCH NO. 3

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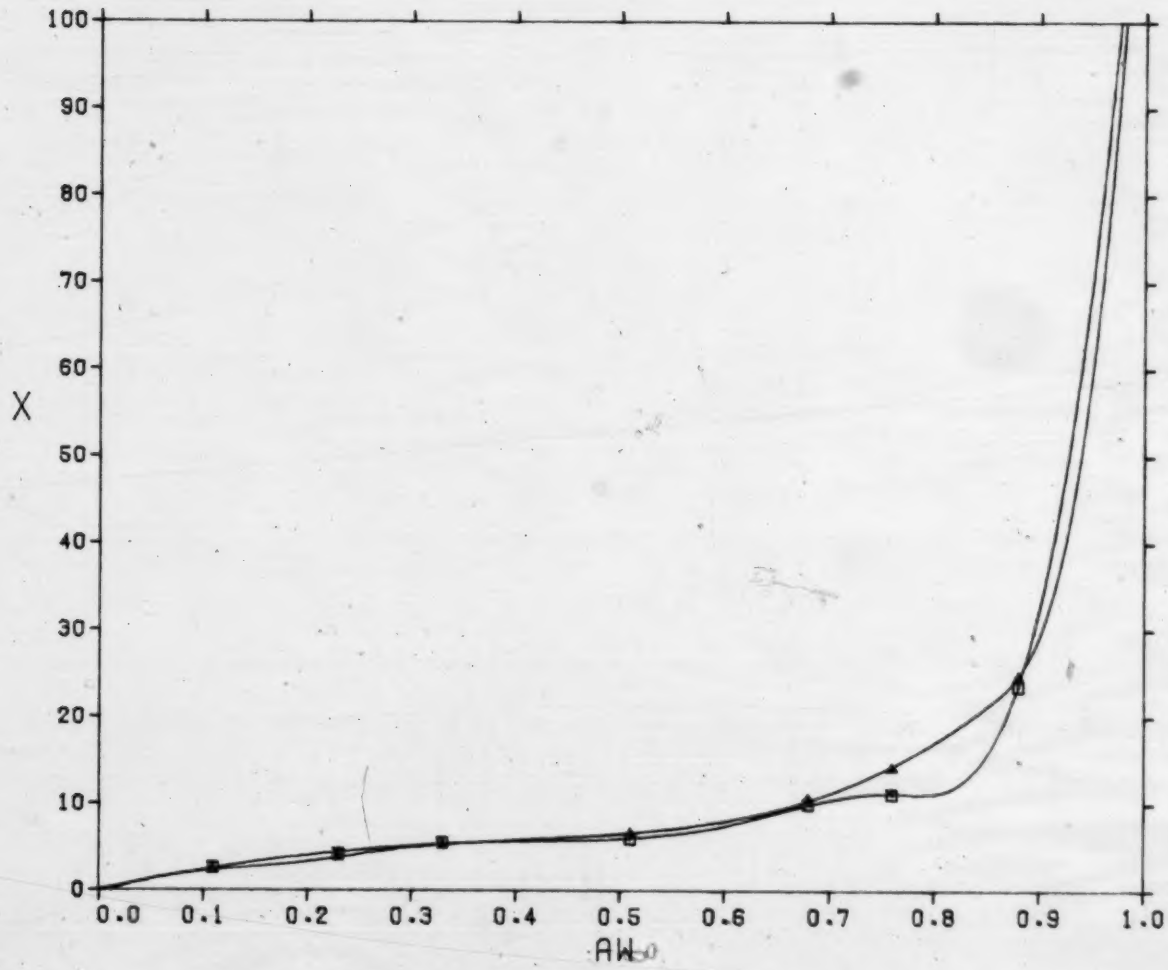

X (KG WATER/100 KG DRY MATTER)

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WHEY PROTEIN

HEAT-PRECIPITATED, FREEZE-DRIED
WASHED, BATCH NO. 3

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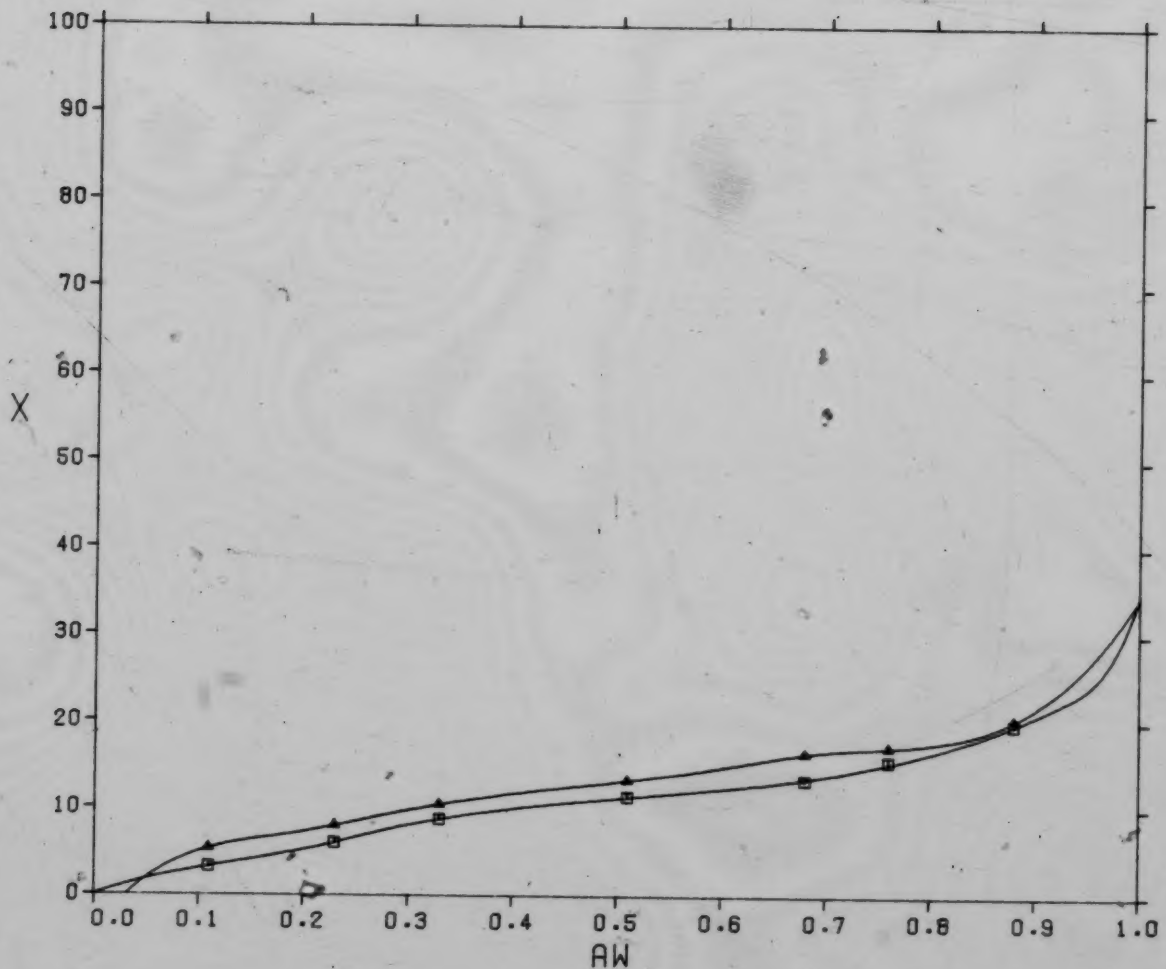

X (KG WATER/100 KG DRY MATTER)

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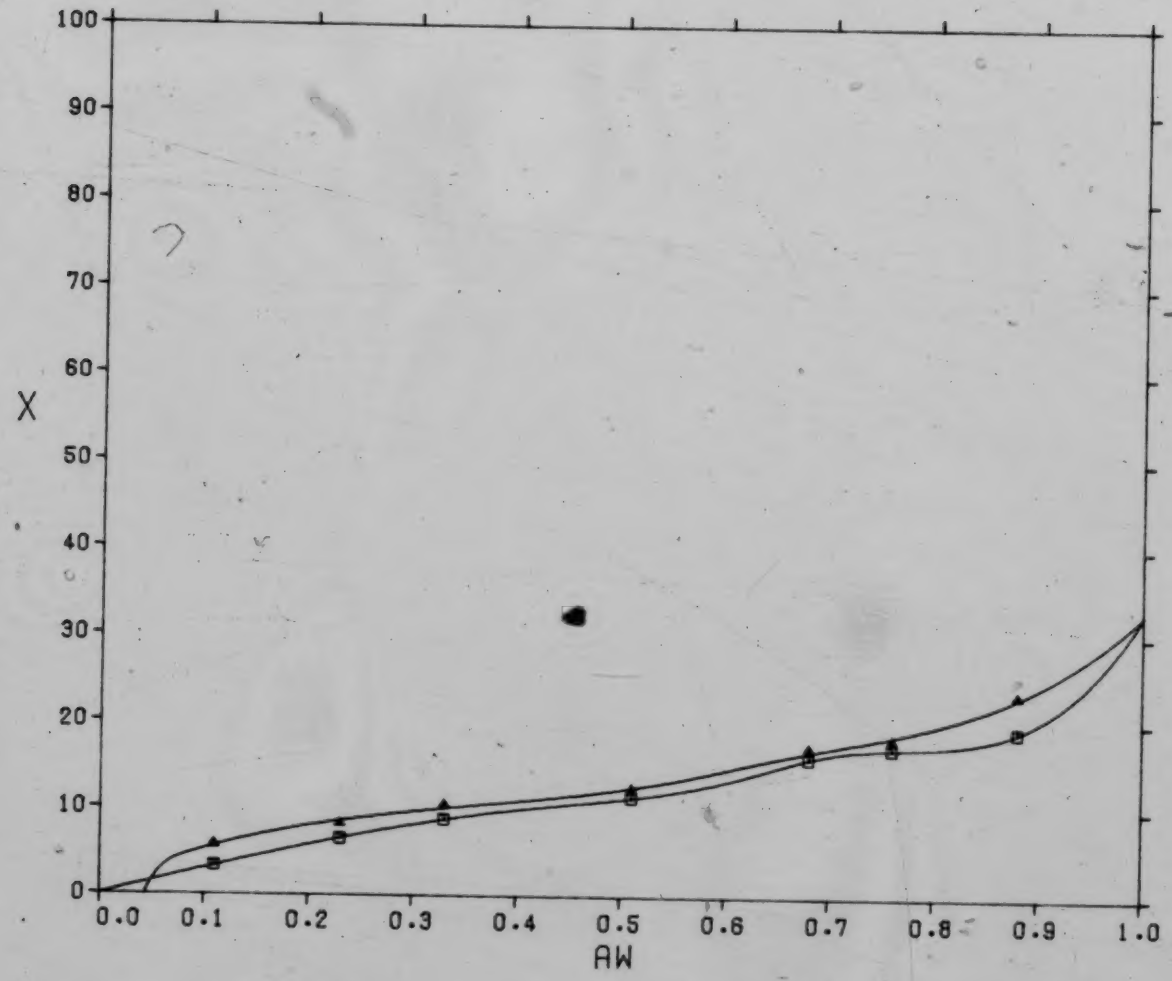
▲ DESORPTION
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 6

PLOT: #0004 17:01:50 APR 5 1978 BY: SORP.C2.F V1

WHEY PROTEIN

HEAT PRECIPITATED FREEZE-DRIED
WASHED, BATCH NO. 3

TEMPERATURE 25.00 DEG. C



X - (KG WATER/100 KG DRY MATTER)

▫ ADSORPTION
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 37

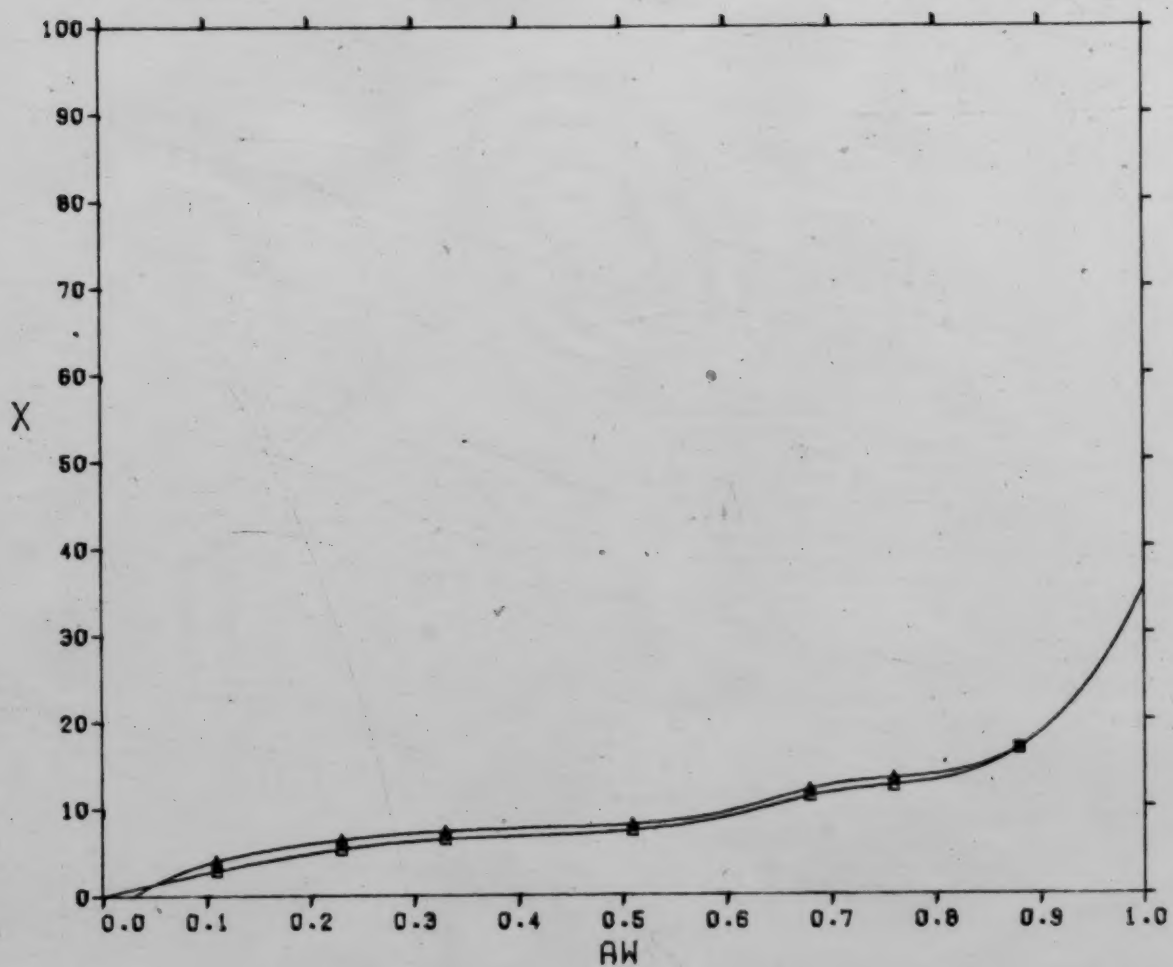
▲ DESORPTION
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 38

PLOT: #0003 14:32:14 MAY 2 1978 BY: SORP.C2.F V1

WHEY PROTEIN

HEAT PRECIPITATED FREEZE DRIED
BATCH NO. 3

TEMPERATURE 40.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

▫ ADSORPTION
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY, 1978
PAGE 45

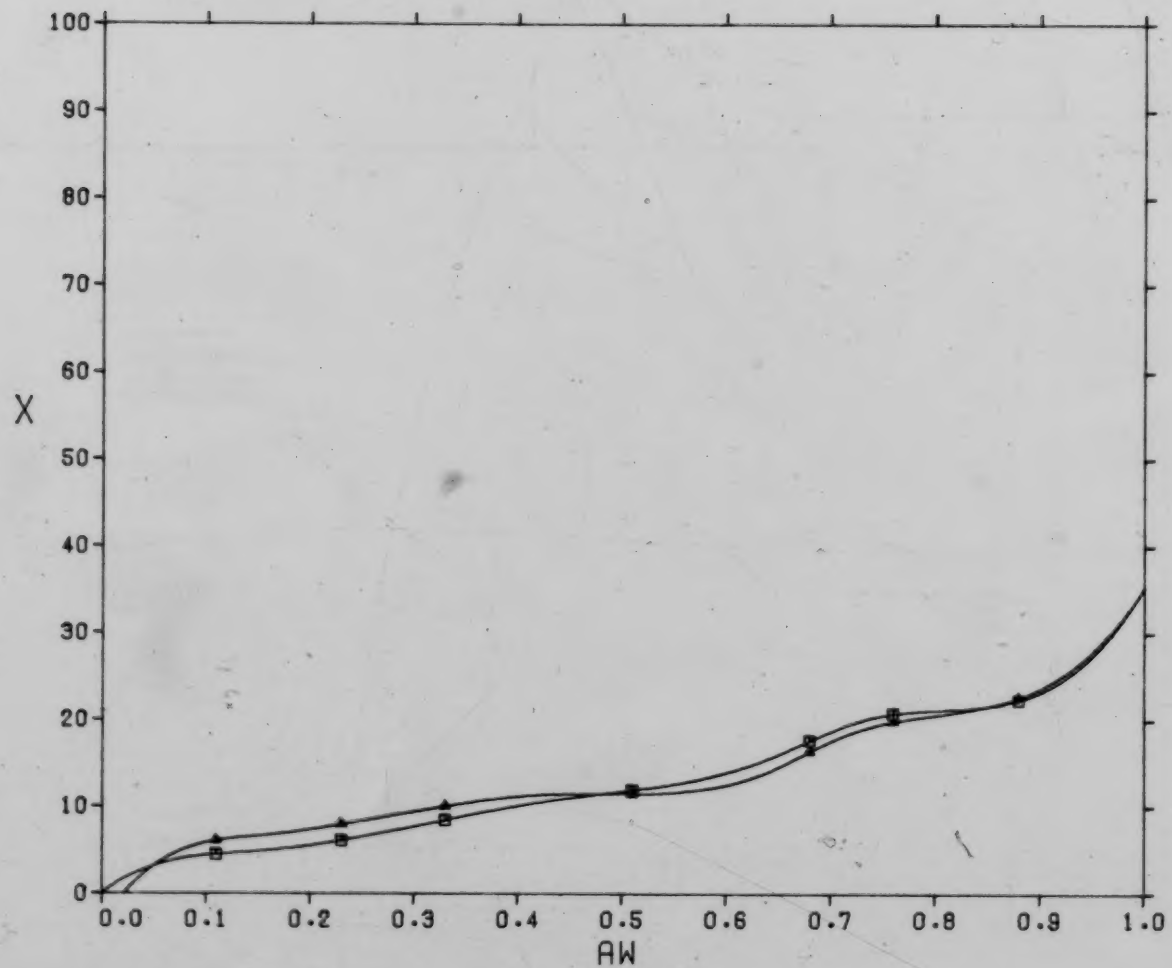
▲ DESORPTION
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY, 1978
PAGE 46

PLOT: #0001 14:51:08 MAY 16 1978 BY: SORP.C2.F V1

WHEY PROTEIN

HEAT PRECIPITATED FREEZE-DRIED
WASHED, BATCH NO. 4

TEMPERATURE 25.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

▫ ADSORPTION
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 33

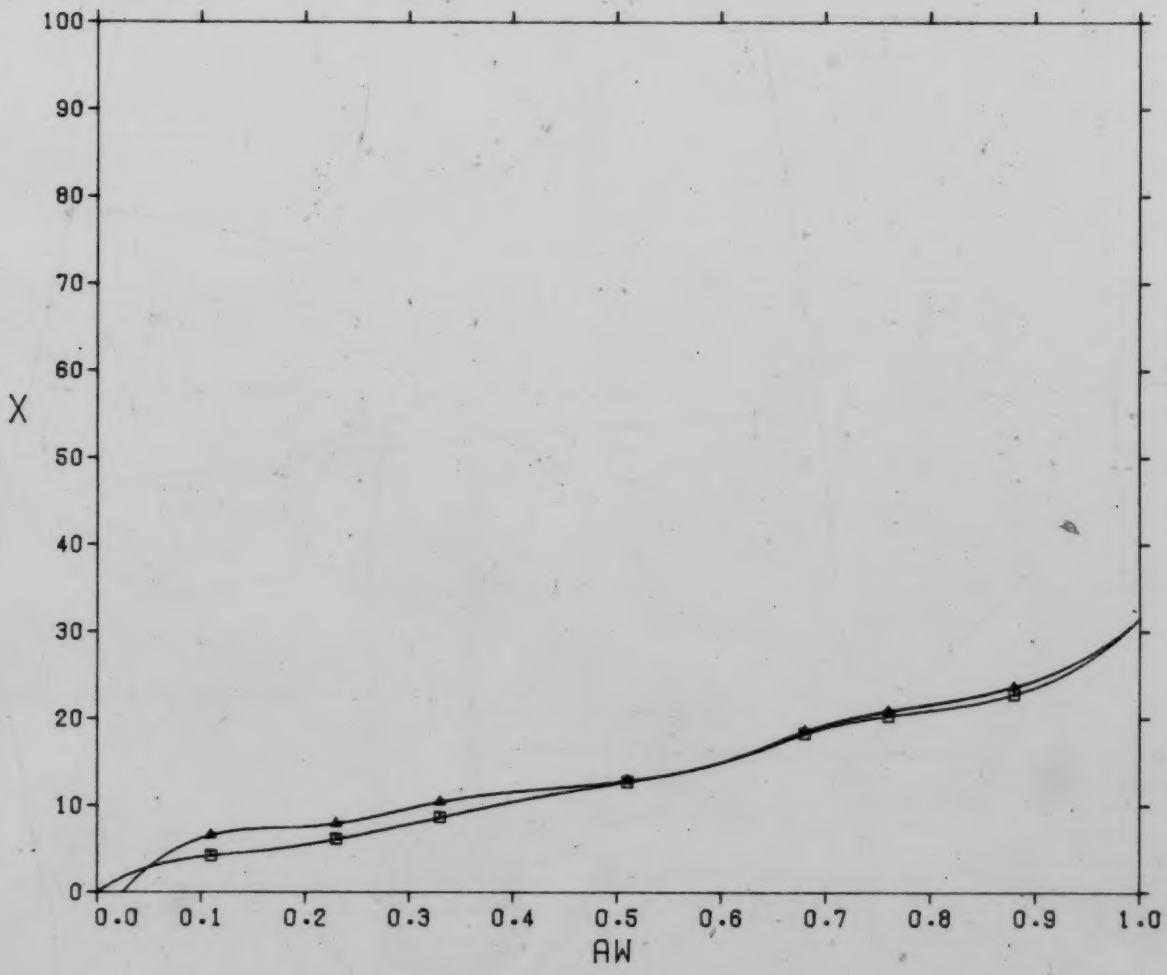
▲ DESORPTION
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 34

PLOT: #0005 14:32:14 MAY 2 1978 BY: SORP.C2.F V1

WHEY PROTEIN

HEAT PRECIPITATED FREEZE-DRIED
WASHED, BATCH NO. 5

TEMPERATURE 25.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

▫ ADSORPTION
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 35

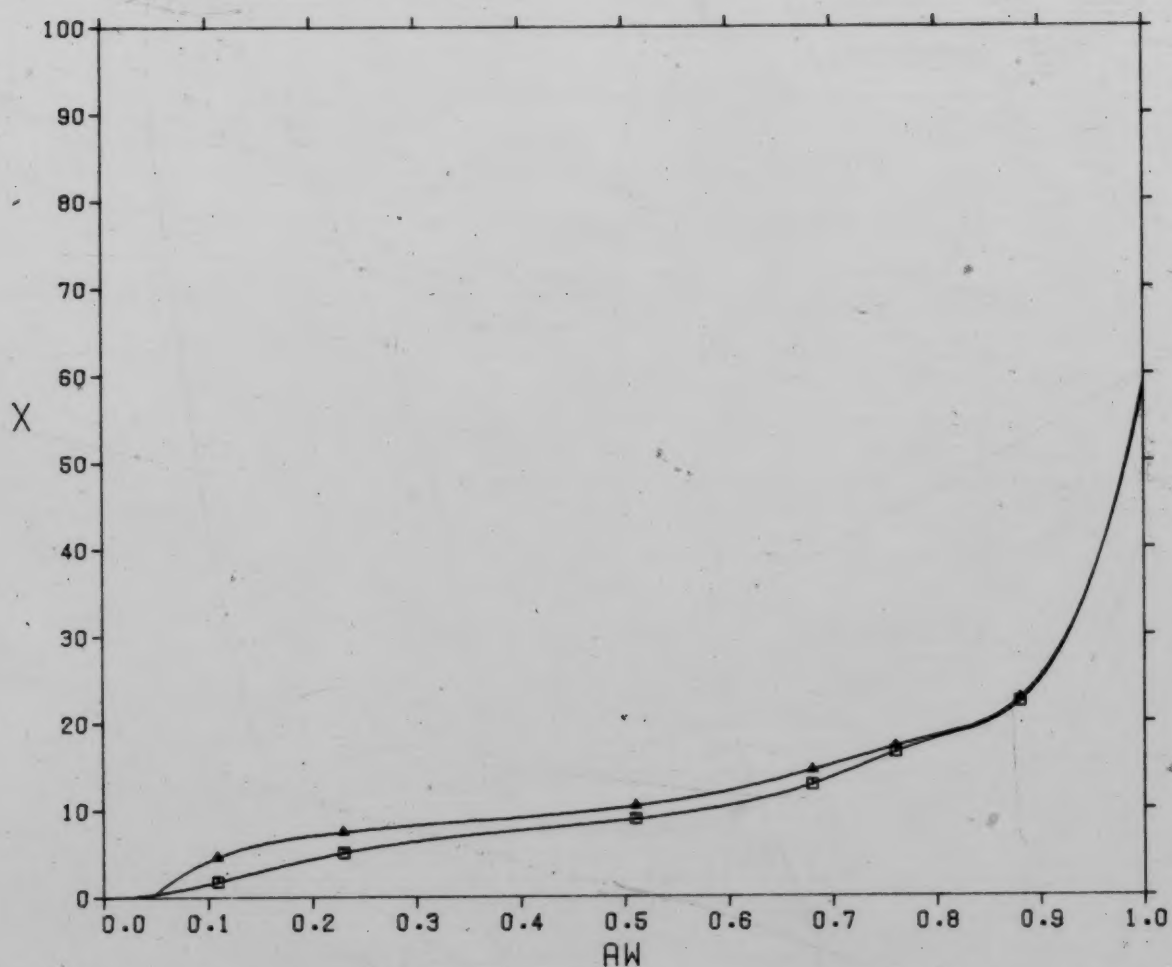
▲ DESORPTION
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 36

PLOT: #0006 14:32:14 MAY 2 1978 BY: SORP.C2.F V1

WHEY PROTEIN POWDER

AIR-DRIED
48 HOURS, 25.0

TEMPERATURE 25.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

▫ ADSORPTION
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 21

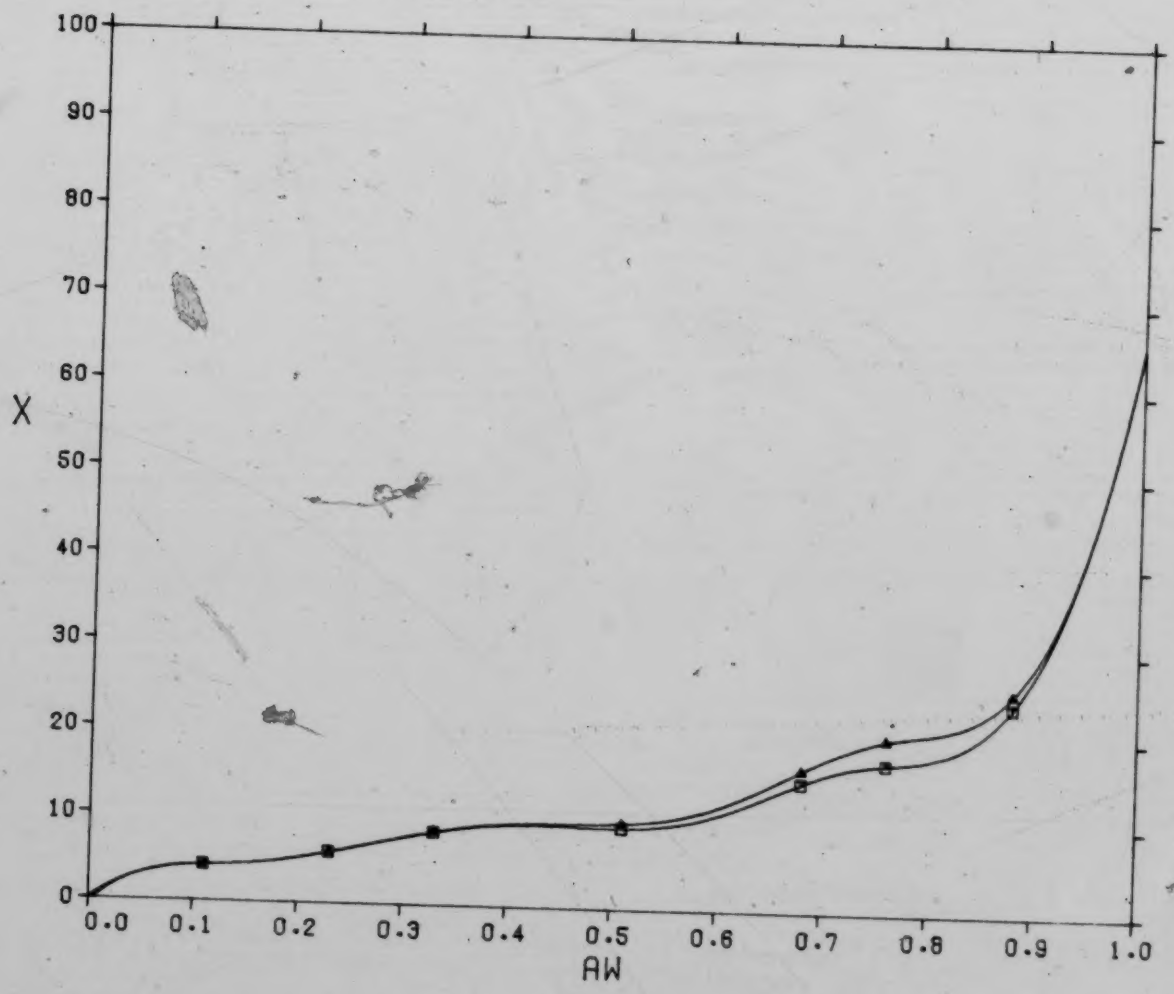
▲ DESORPTION
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 22

PLOT: #0010 17:01:50 APR 5 1978 BY: SORP.C2.F V1

WHEY PROTEIN

HEAT PRECIPITATED VACUUM DRIED
36 HOURS, 60.0, 60 MESH

TEMPERATURE 25.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

▫ ADSORPTION
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 43

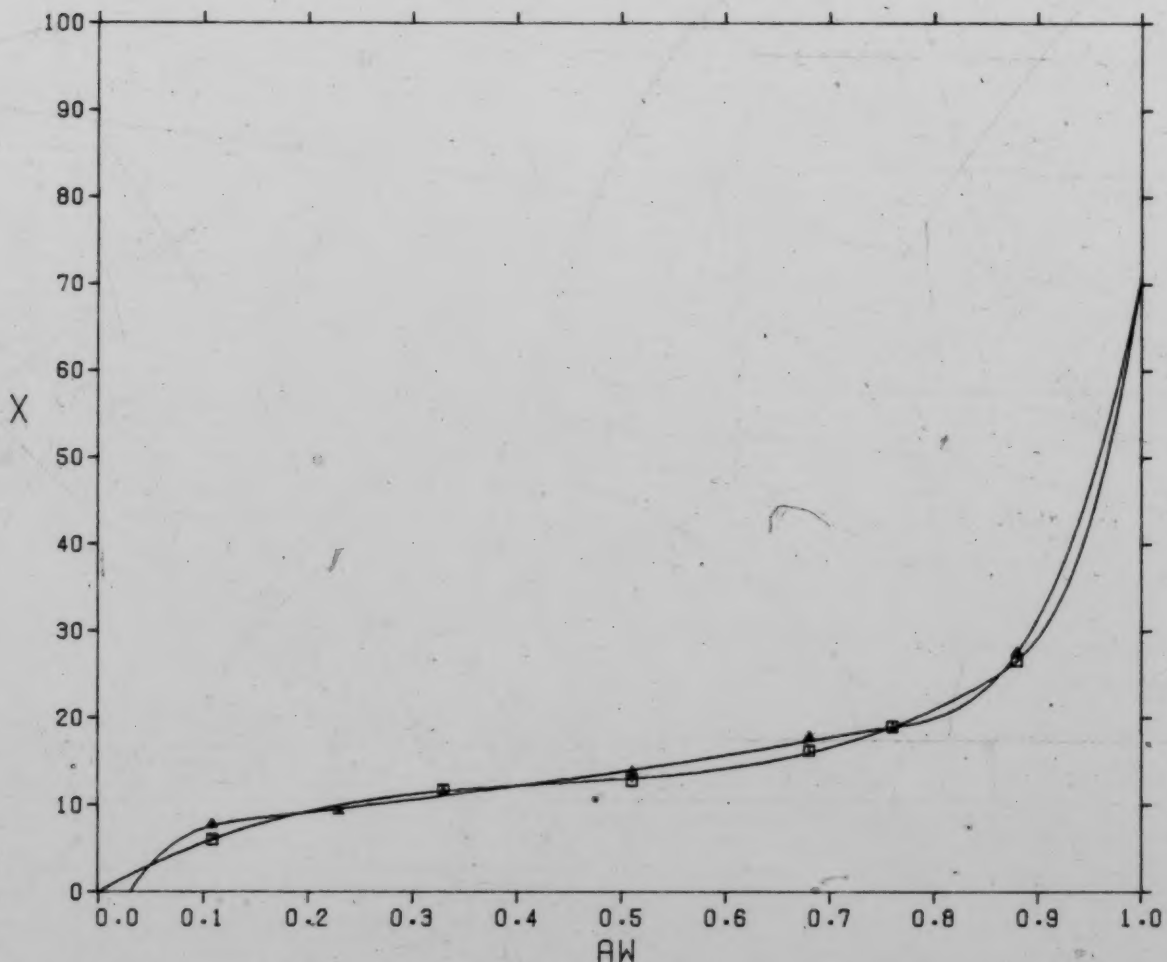
▲ DESORPTION
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 44

PLOT: #0007 14:32:14 MAY 2 1978 BY: SORP.C2.F V1

WHEY PROTEIN POWDER

DIALYSED. UNDENATURED
FREEZE-DRIED

TEMPERATURE 25.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

□ ADSORPTION
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 17

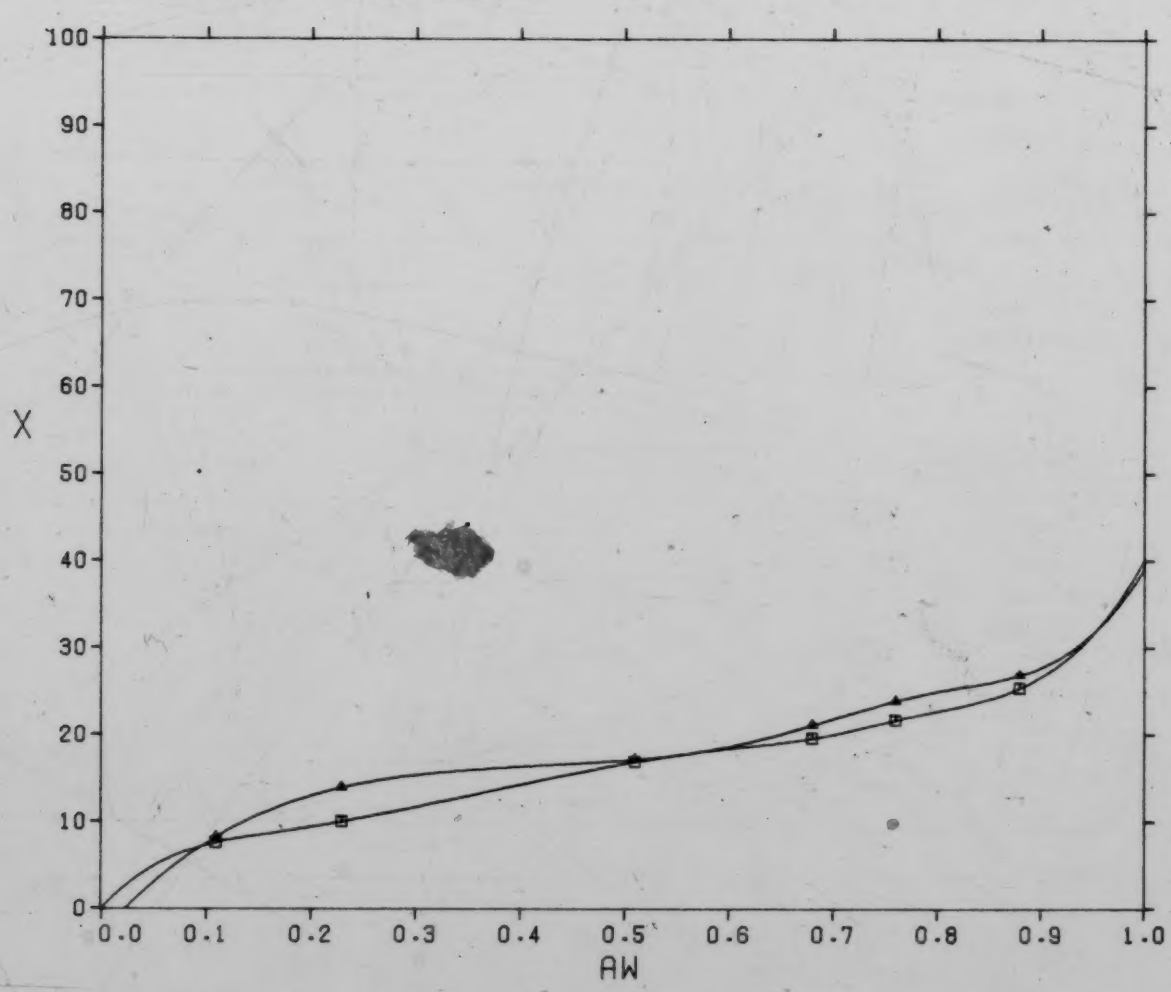
▲ DESORPTION
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 18

PLOT: #0002 09:21:02 APR 6 1978 BY: SORP.C2.F V1

WHEY PROTEIN POWDER

DIALYSED, DENATURED
FREEZE-DRIED

TEMPERATURE 25.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

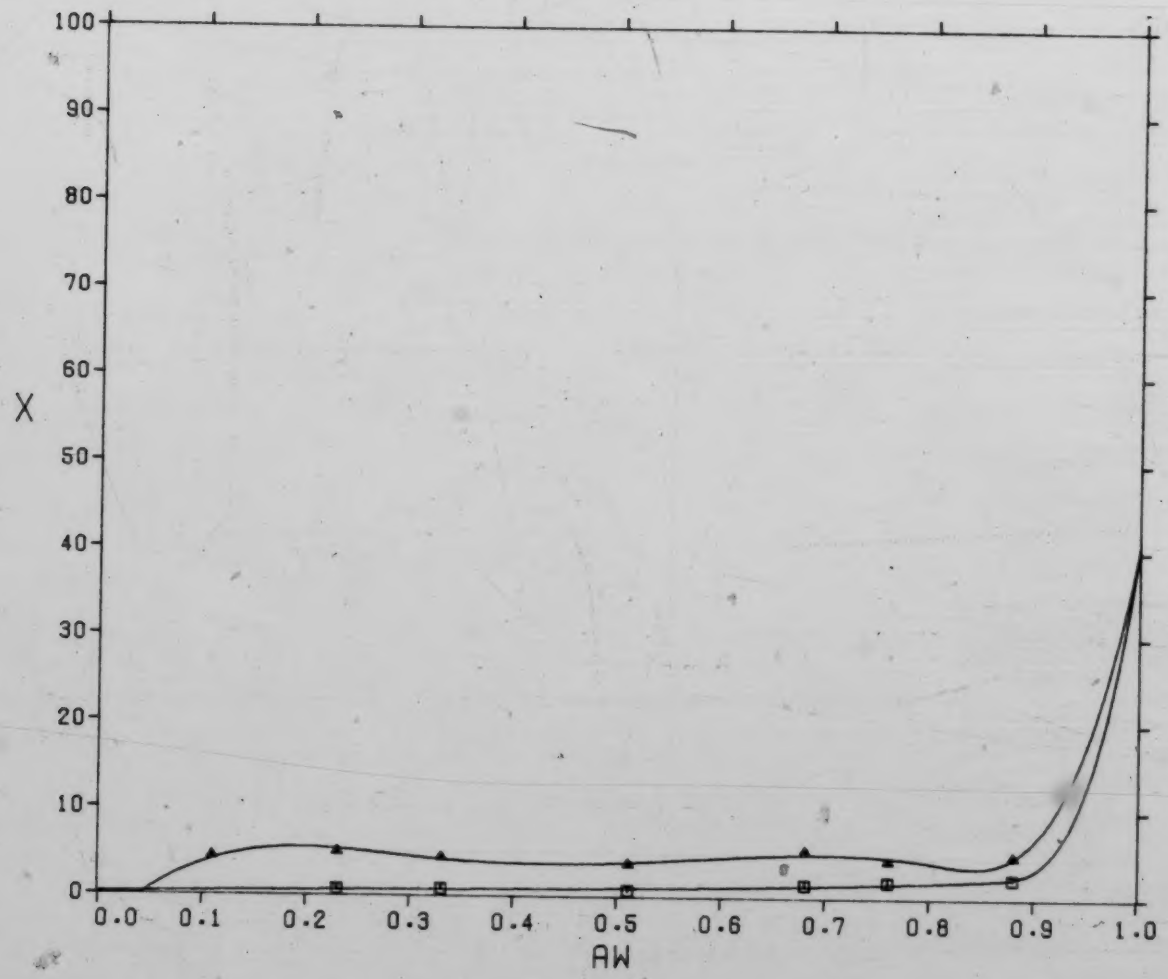
□ ADSORPTION
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 19

▲ DESORPTION
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 20

PLOT: #0001 09:21:02 APR 6 1978 BY SORP.C2.F V1

LACTOSE GLASS

TEMPERATURE 25.00 DEG. C



X (KG WATER/100 KG DRY MATTER)

▫ ADSORPTION
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 25

▲ DESORPTION
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
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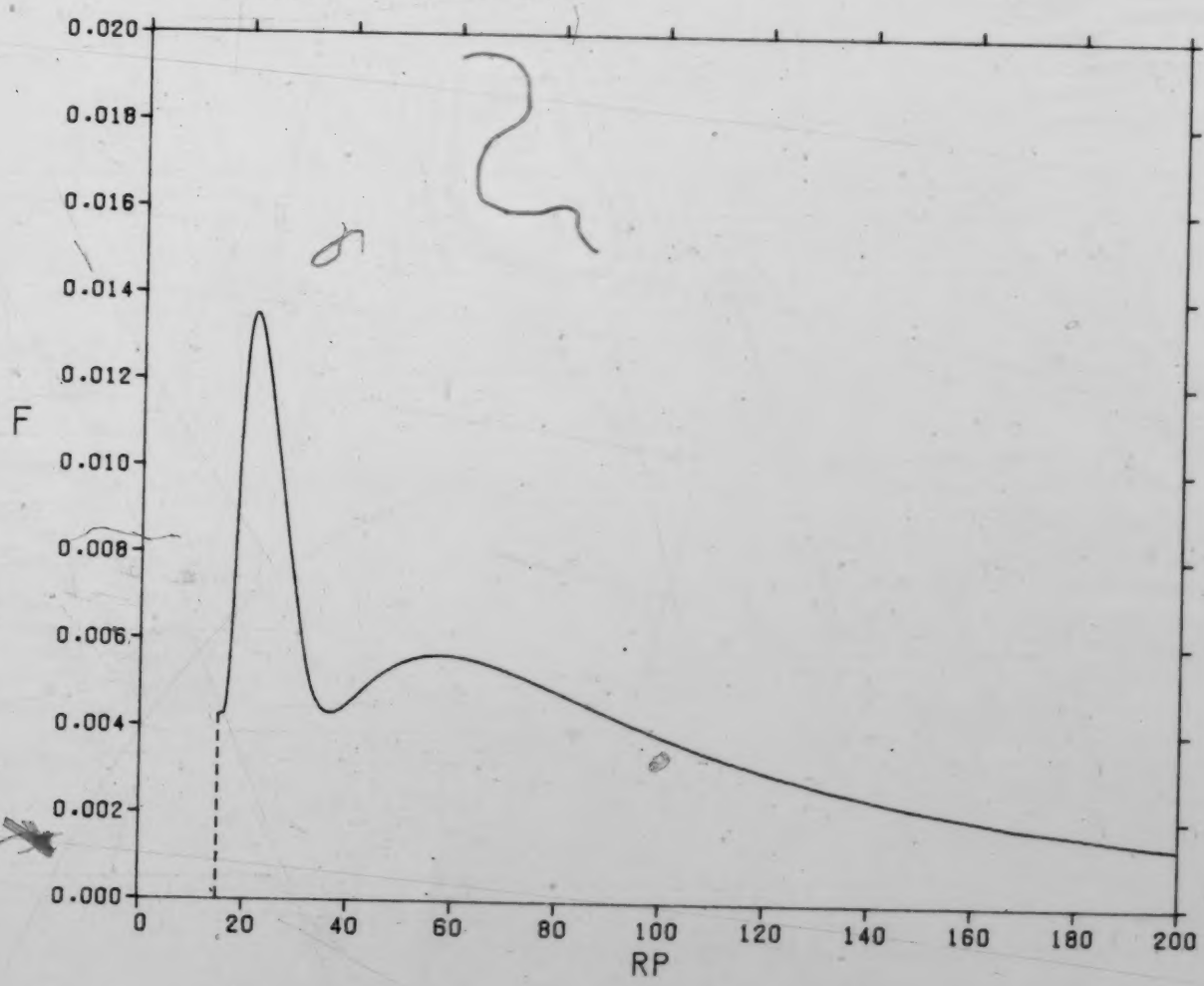
PLOT: #0007 09:21:02 APR 6 1978 BY: SORP.C2.F V1

WHEY PROTEIN

HEAT-PRECIPITATED, FREEZE-DRIED, BATCH NO. 1
UNWASHED

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

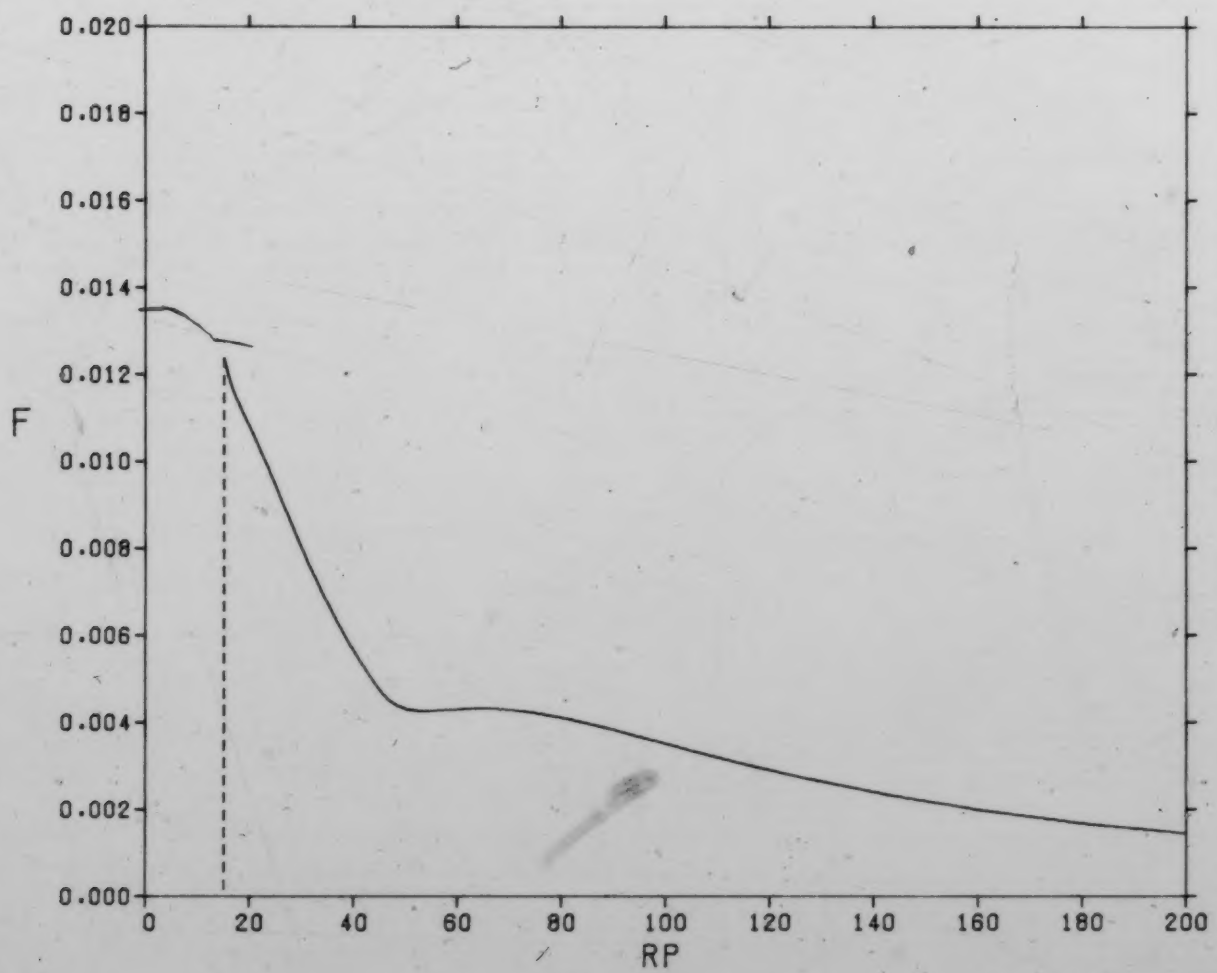
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 13

PLOT: #0017 09:28:40 APR 7 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT-PRECIPITATED, FREEZE-DRIED
UNWASHED, BATCH NO. 2

TEMPERATURE 12.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

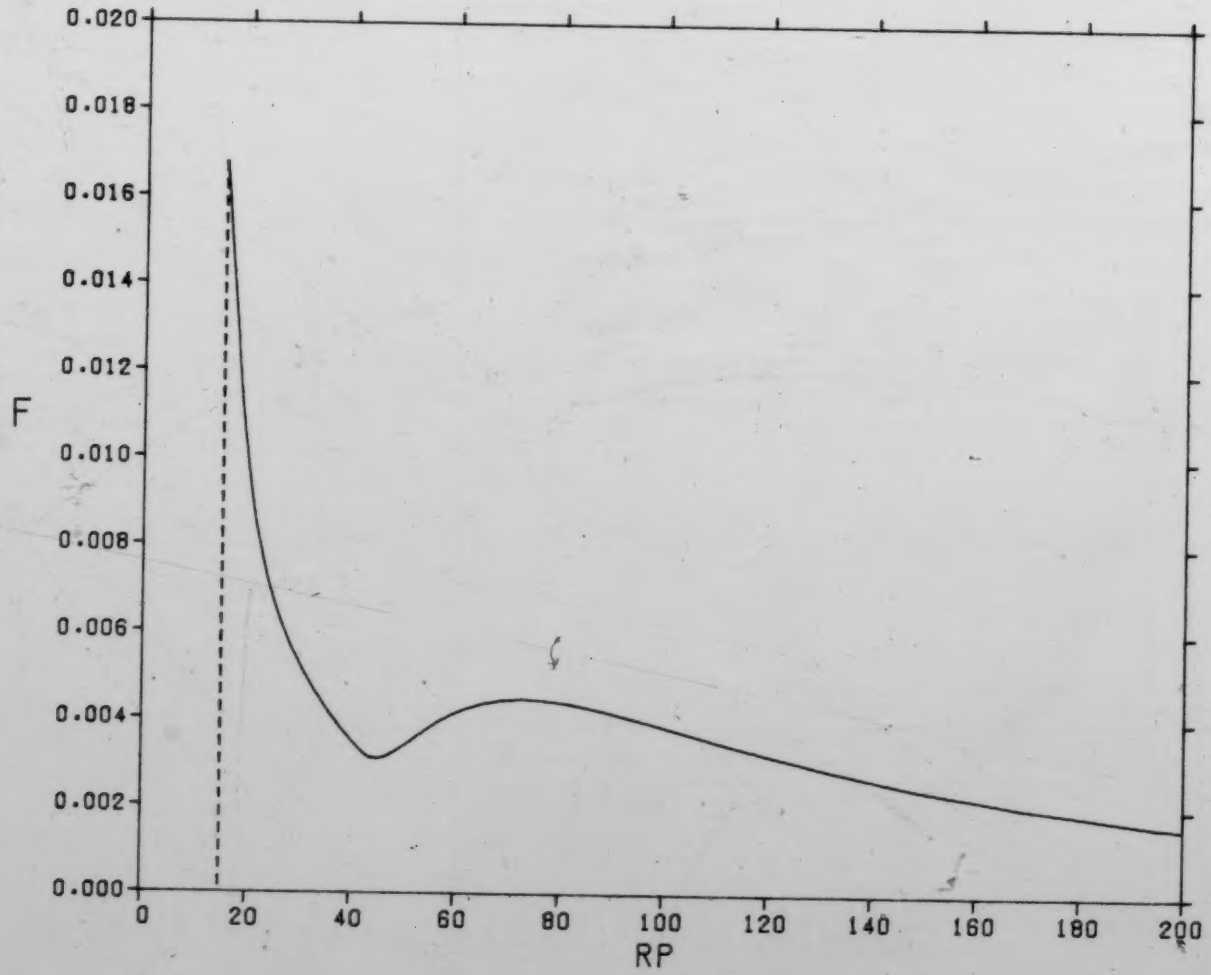
ADSORPTION DATA
ROBERT I.W. GREIG
LAB BOOK # . MAY-SEPT.. 1977
PAGE 3

PLOT: #0001 16:01:17 OCT 19 1977 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT-PRECIPITATED, FREEZE-DRIED
UNWASHED, BATCH NO. 2

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

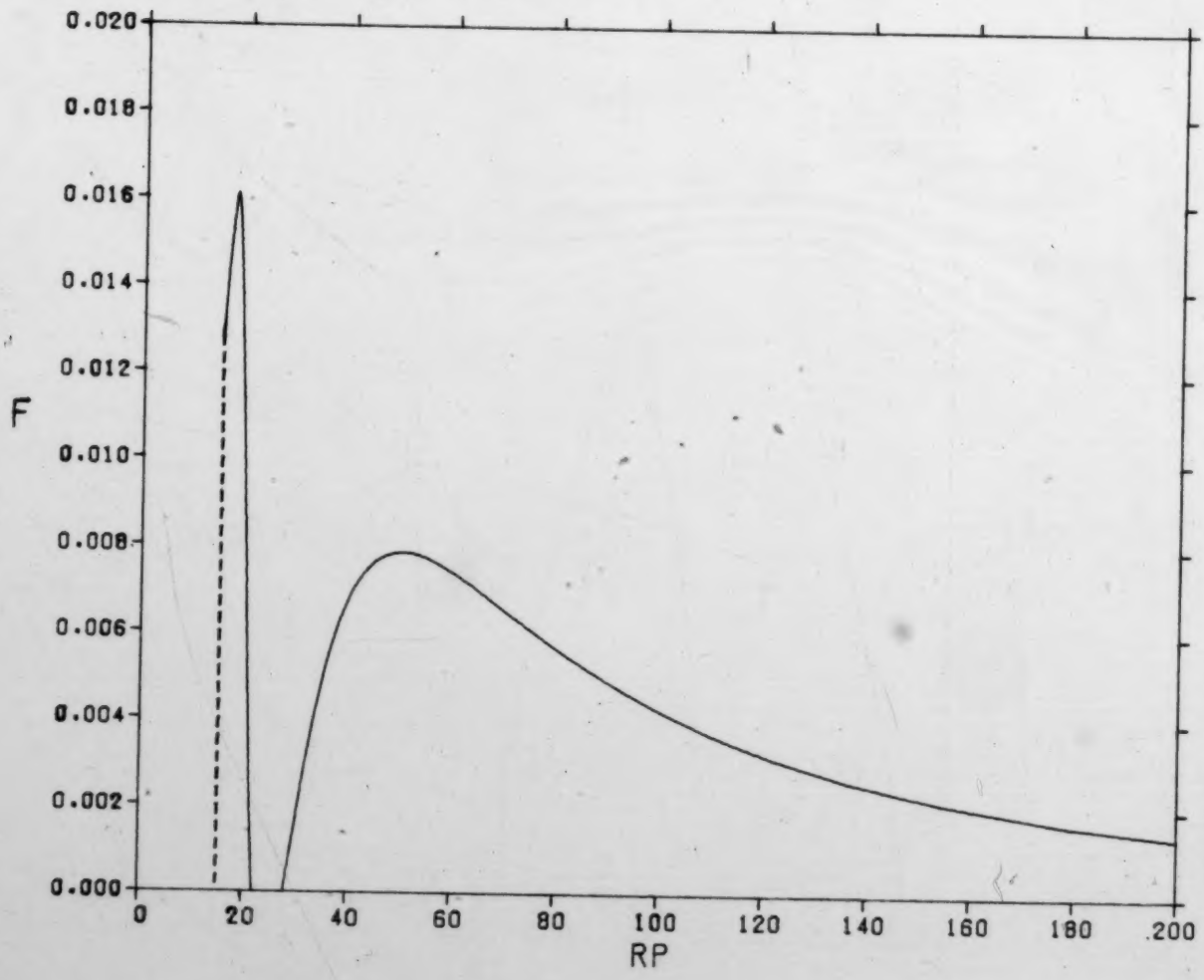
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, MAY-SEPT., 1977
PAGE 3

PLOT: #0001 11:30:50 SEP 29 1977 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT-PRECIPITATED, FREEZE-DRIED
UNWASHED, BATCH NO. 2

TEMPERATURE 40.00 DEG. C



RP - PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

ADSORPTION DATA
ROBERT I. W. CREIG
LAB BOOK#1, MAY-SEPT., 1977
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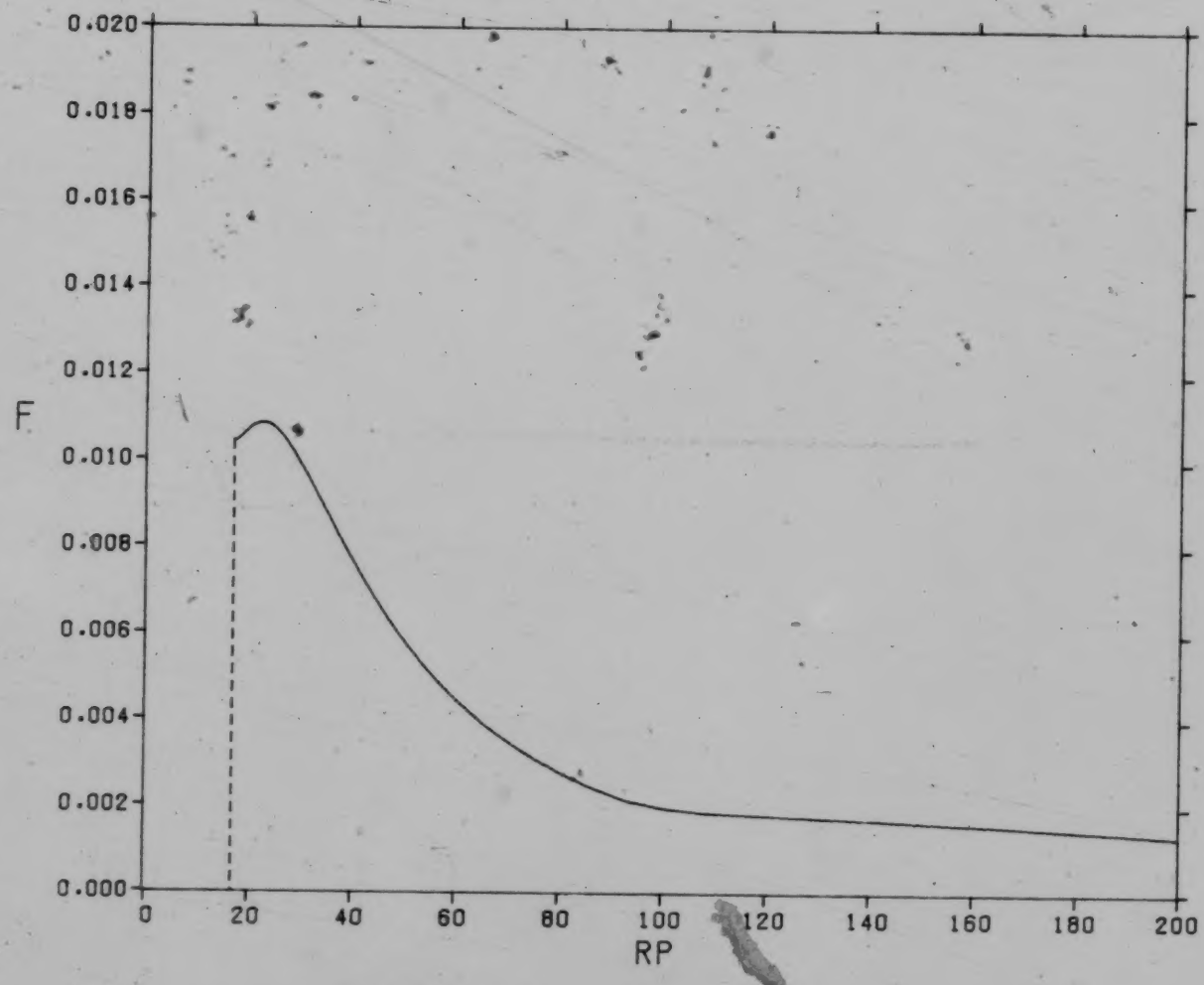
PLOT: #0005 11:30:50 SEP 29 1977 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT-PRECIPITATED, FREEZE-DRIED
WASHED, BATCH NO. 3

TEMPERATURE 12.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
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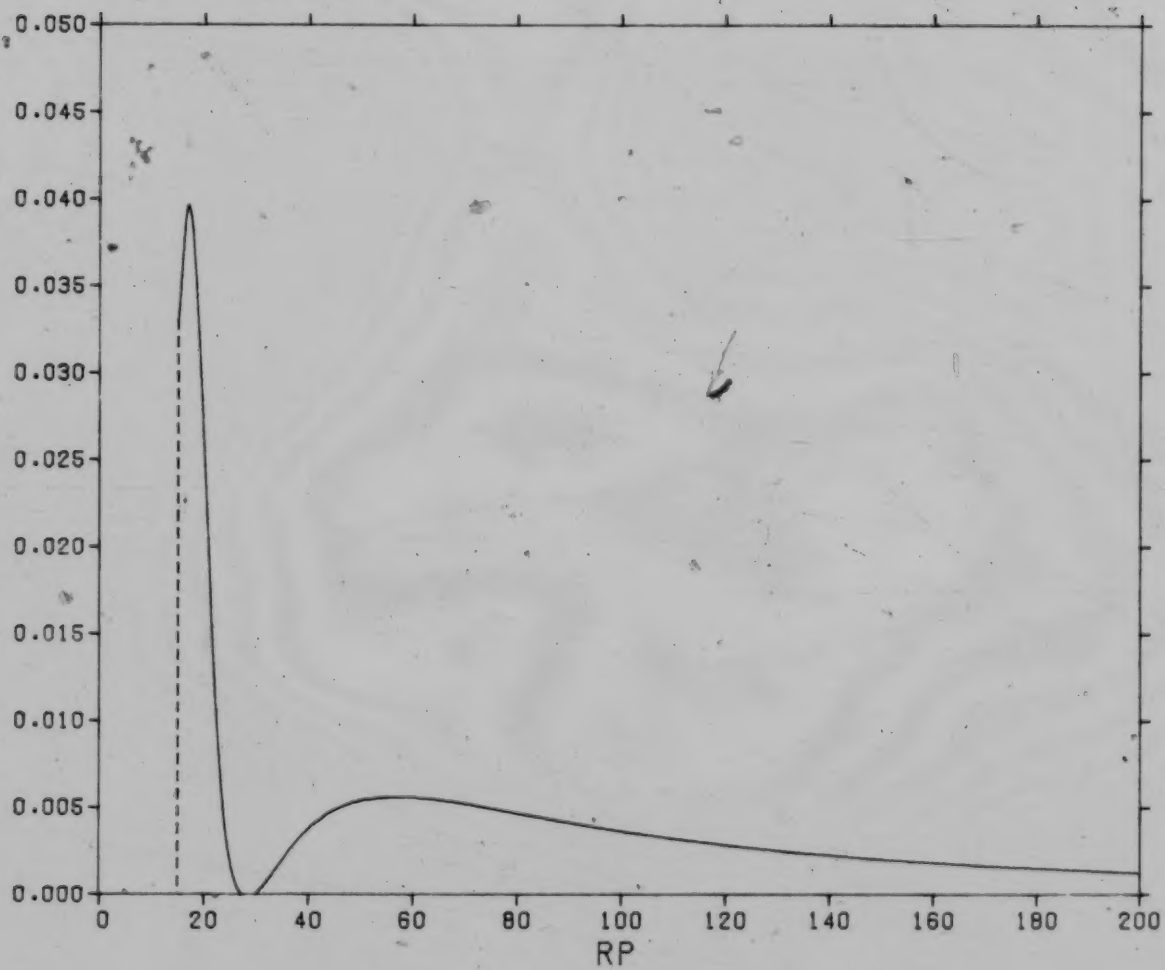
PLOT: #0001 23:13:55 APR 6 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT PRECIPITATED FREEZE-DRIED
WASHED, BATCH NO. 3

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
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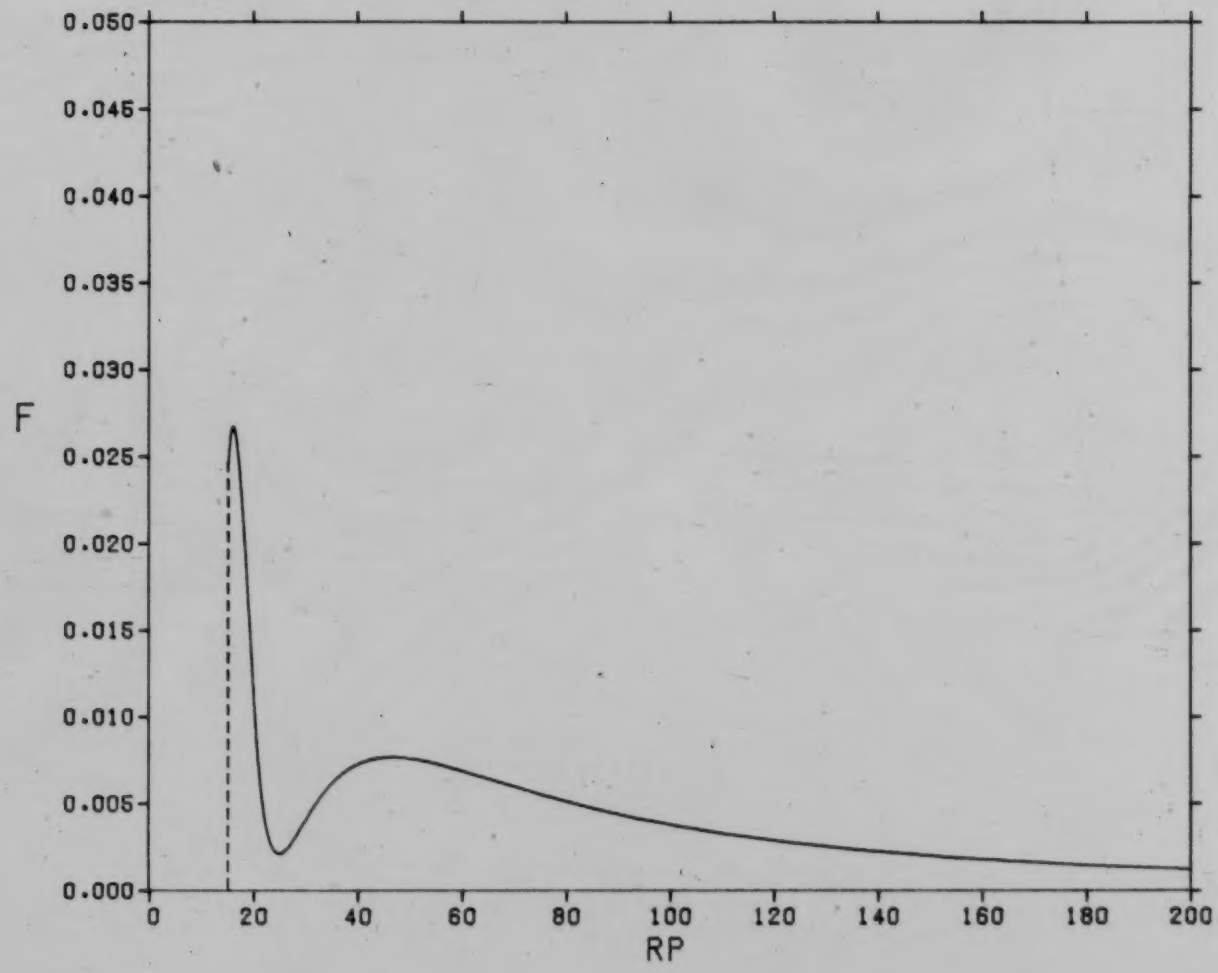
PLOT: #0009 10:31:01 MAY 5 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT PRECIPITATED FREEZE DRIED
BATCH NO. 3

TEMPERATURE 40.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

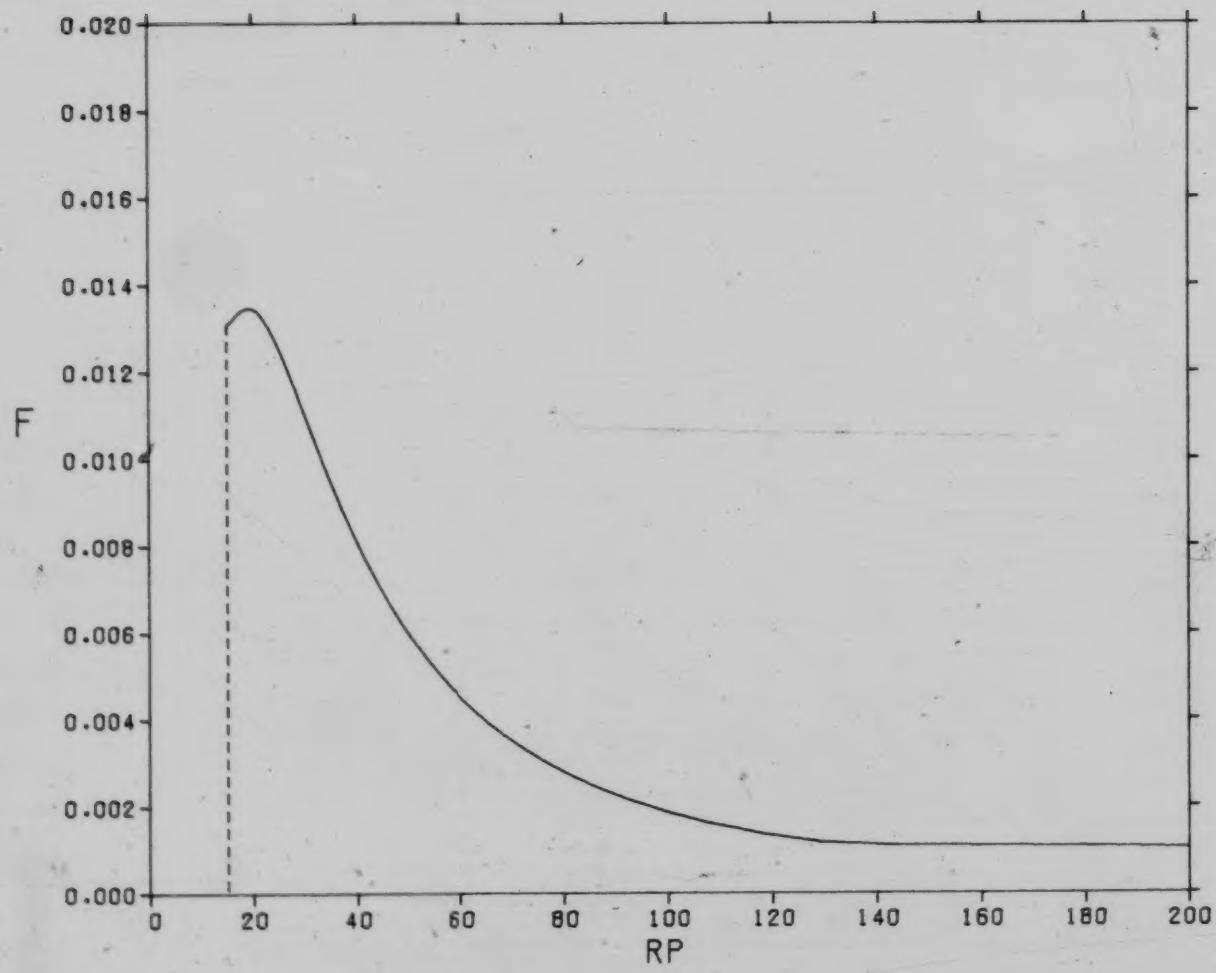
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY, 1978
PAGE 45

PLOT: #0001 13:07:26 MAY 17 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT-PRECIPITATED, SPRAY-DRIED
WASHED, BATCH NO. 3

TEMPERATURE 12.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

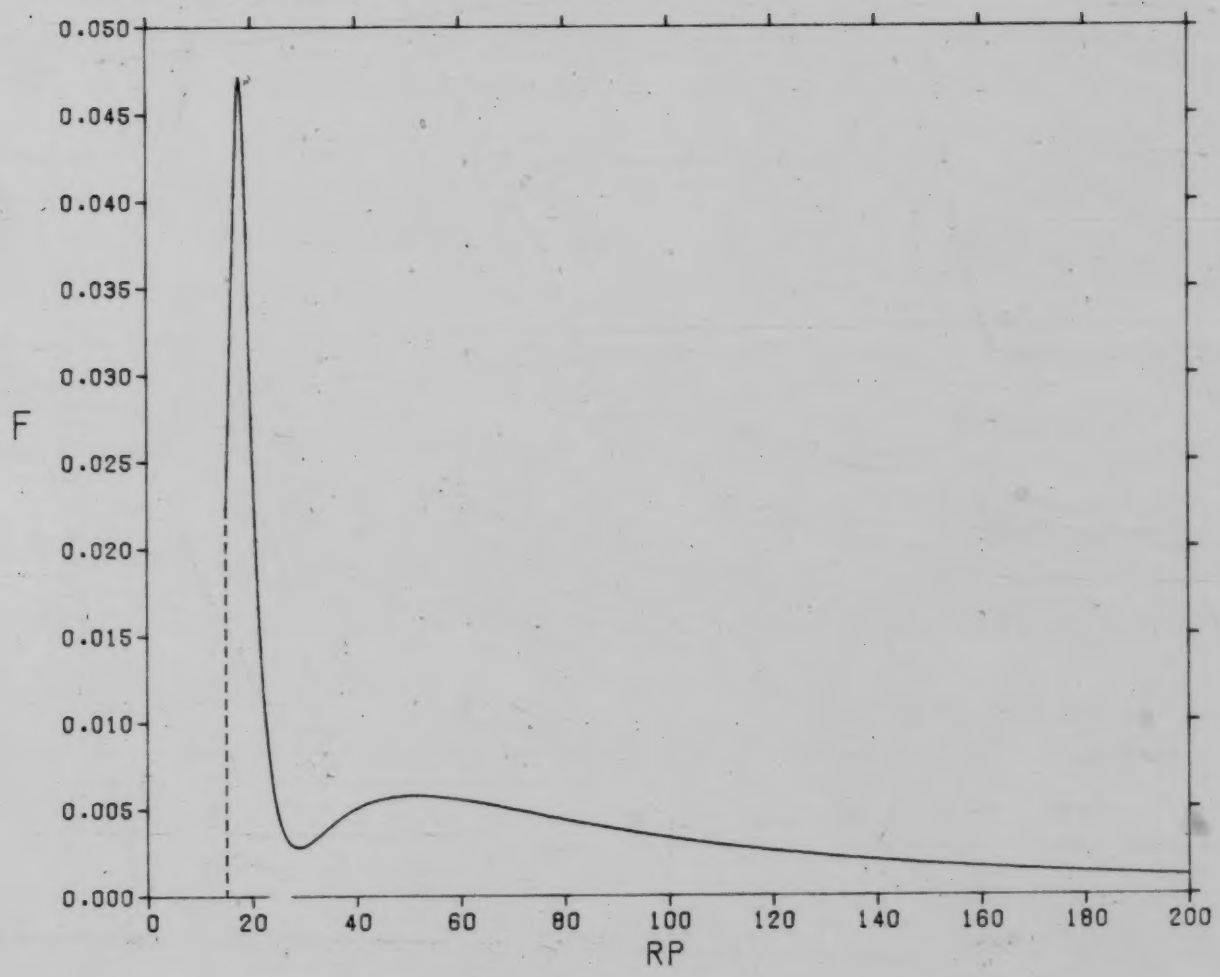
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 8

PLOT: #0017 23:13:55 APR 6 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT PRECIPITATED SPRAY DRIED
WASHED, BATCH NO. 3

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

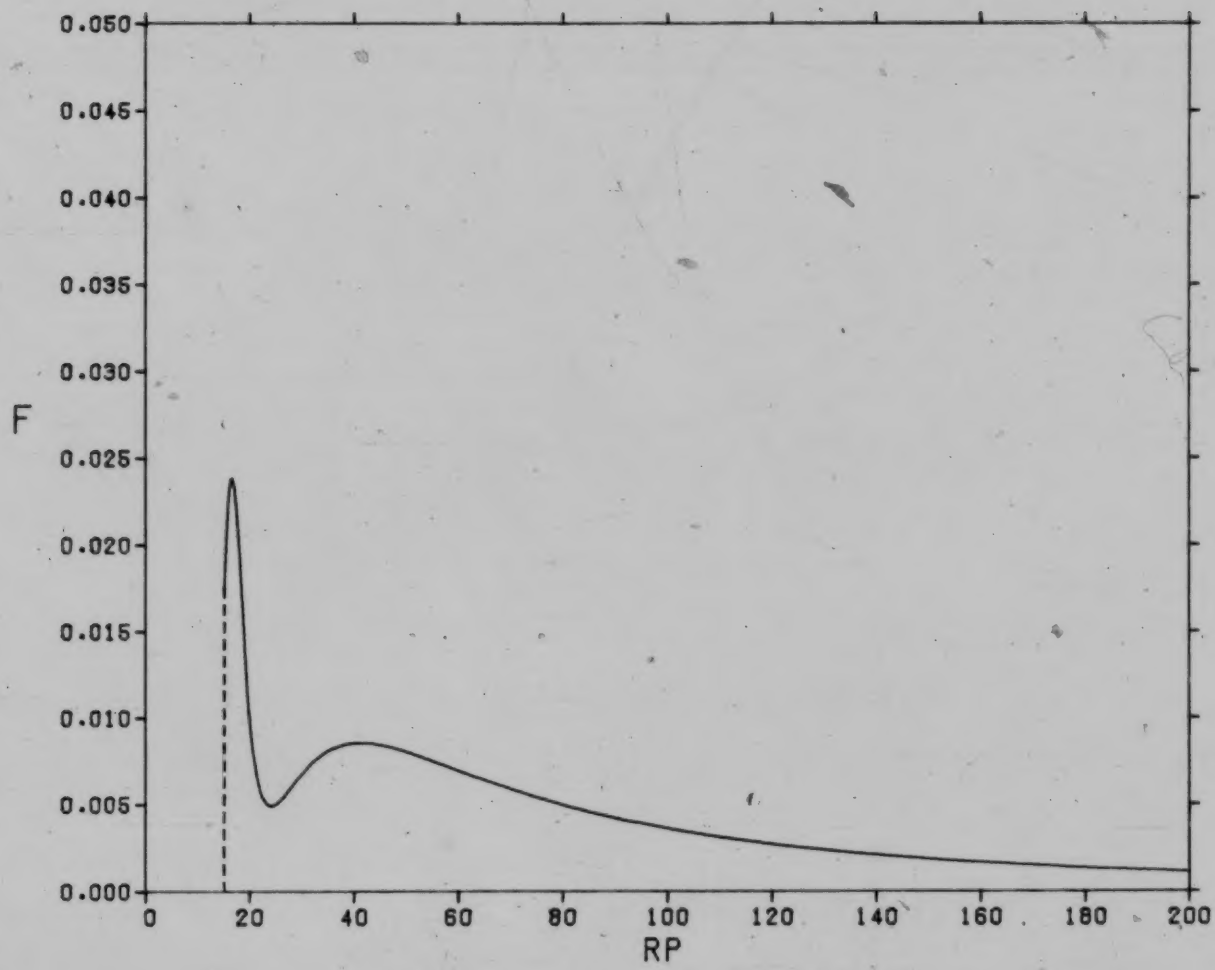
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 39

PLOT: #0013 10:31:01 MAY 5 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT PRECIPITATED SPRAY DRIED
BATCH NO. 3

TEMPERATURE 40.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY, 1978
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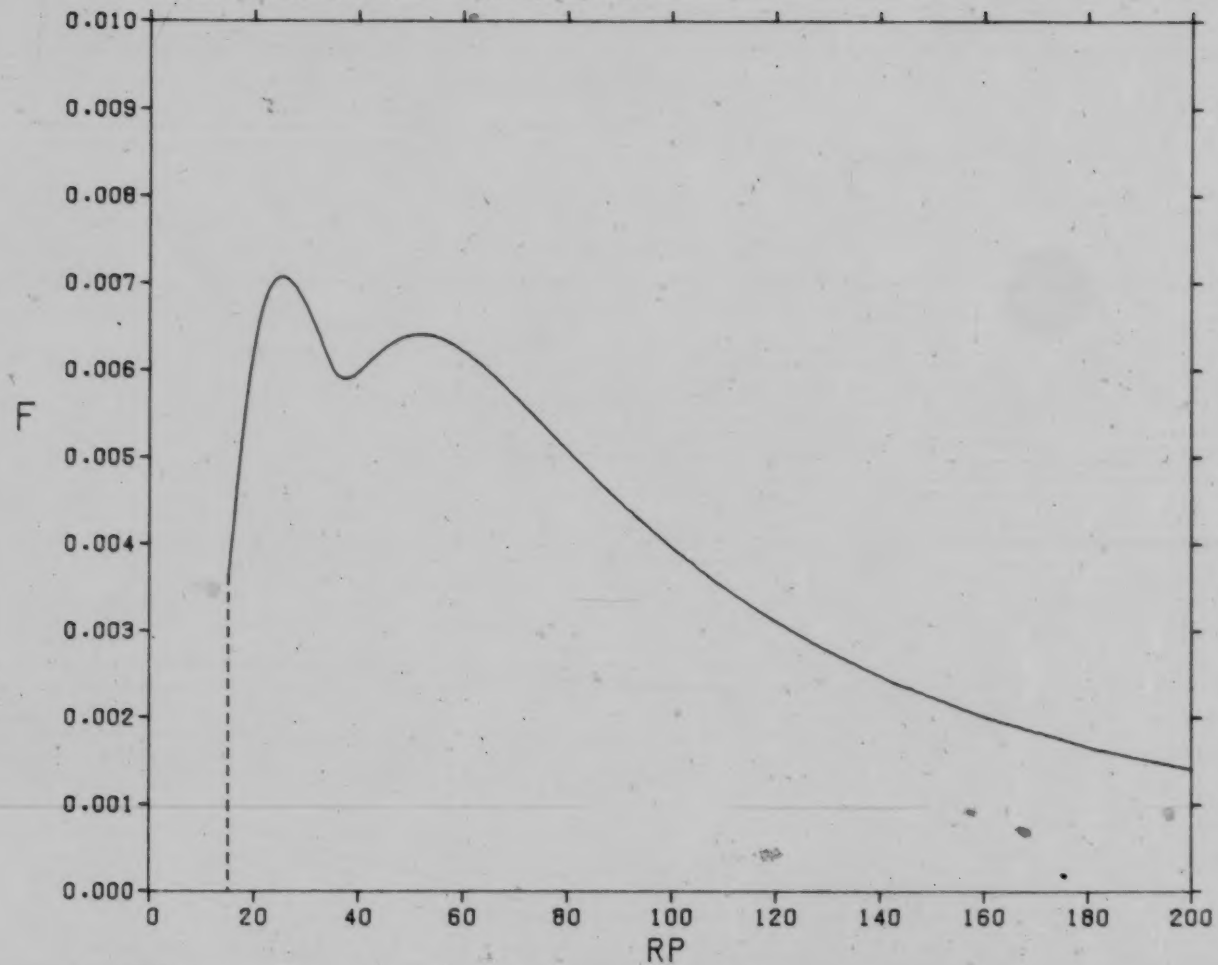
PLOT: #0009 13:07:26 MAY 17 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT-PRECIPITATED, DRUM-DRIED
WASHED, BATCH NO. 3

TEMPERATURE 12.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

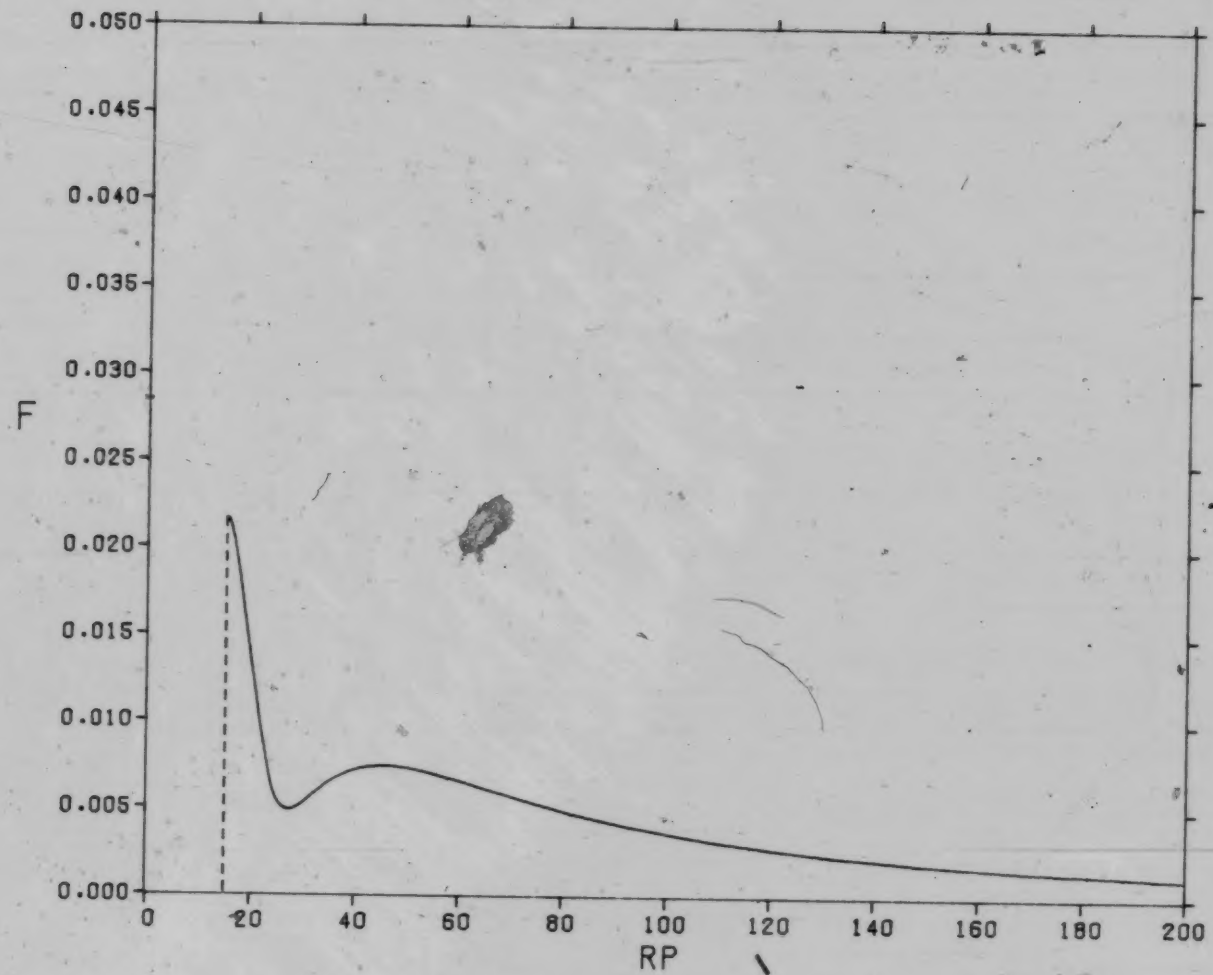
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 7

PLOT: #0009 23:13:55 APR 6 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT-PRECIPITATED, DRUM-DRIED
WASHED, BATCH NO. 3

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

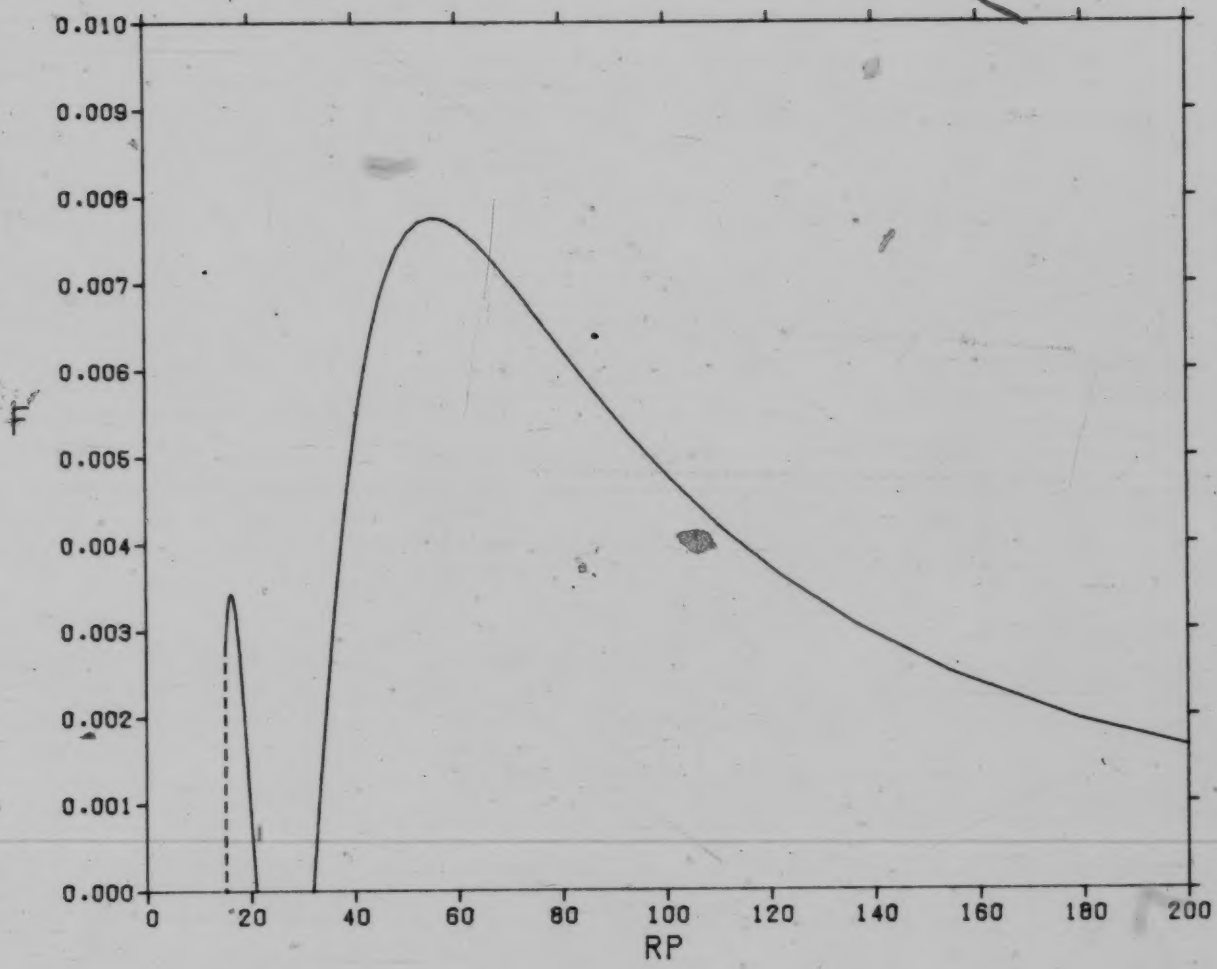
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 7

PLOT: #0013 23:13:55 APR 6 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT PRECIPITATED DRUM DRIED
BATCH NO. 3

TEMPERATURE 40.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

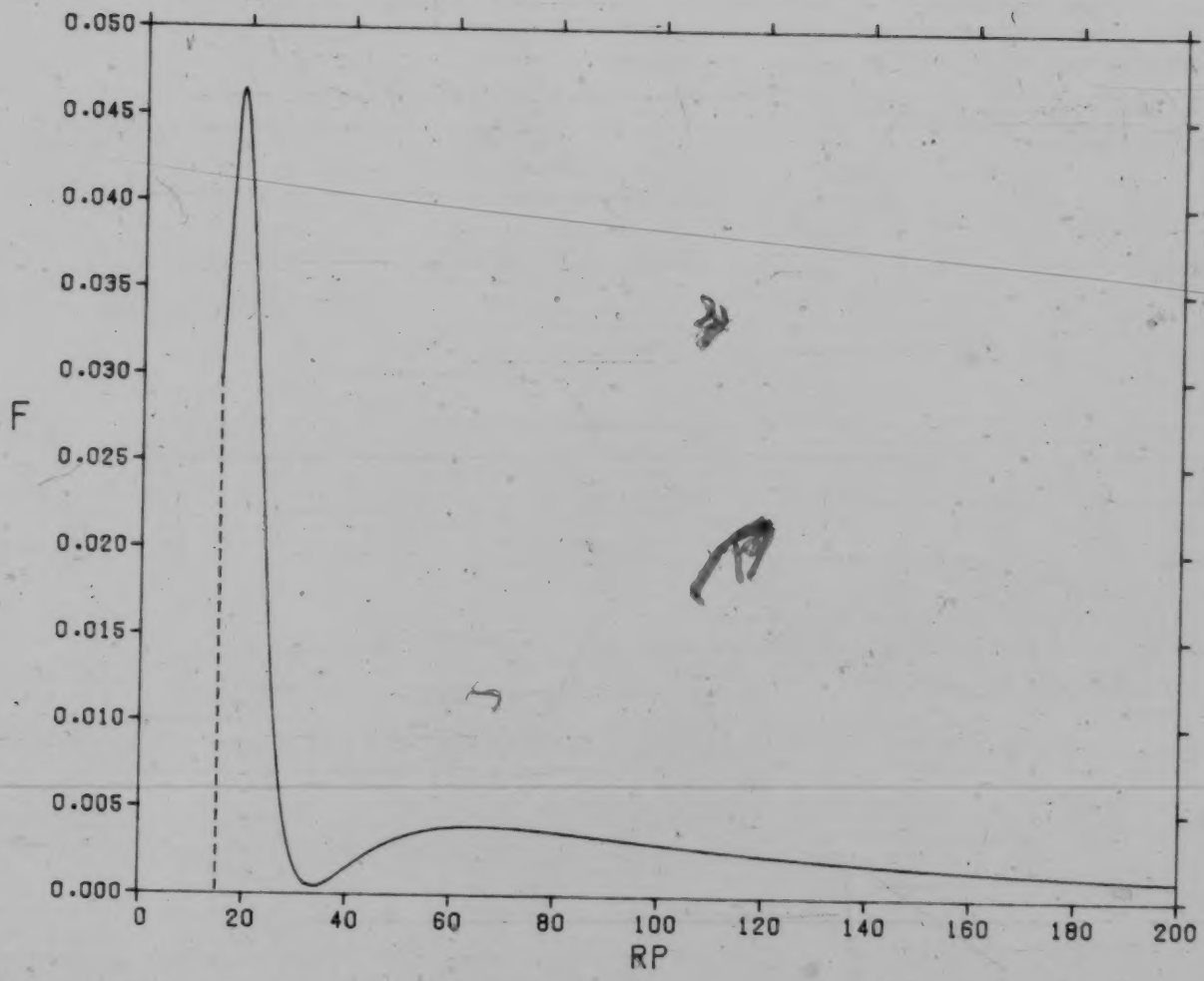
ADSORPTION DATA
ROBERT I. [redacted] GREIG
LAB BOOK#1, APR.-MAY, 1978
PAGE 47

PLOT: #0005 13:07:26 MAY 17 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT PRECIPITATED FREEZE-DRIED
WASHED, BATCH NO. 4

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

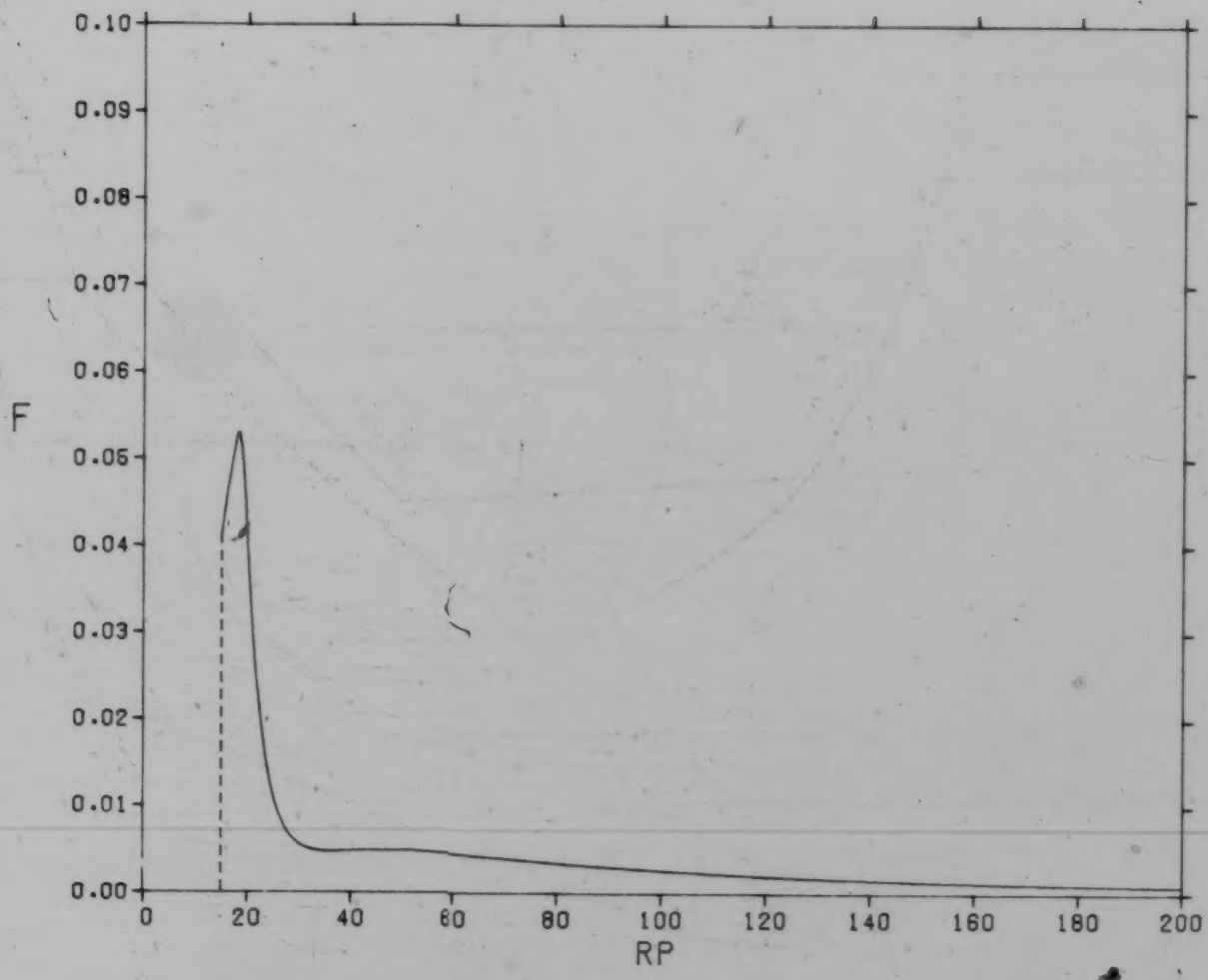
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 33

PLOT: #0017 10:31:01 MAY 5 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT PRECIPITATED FREEZE-DRIED
WASHED, BATCH NO. 5

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

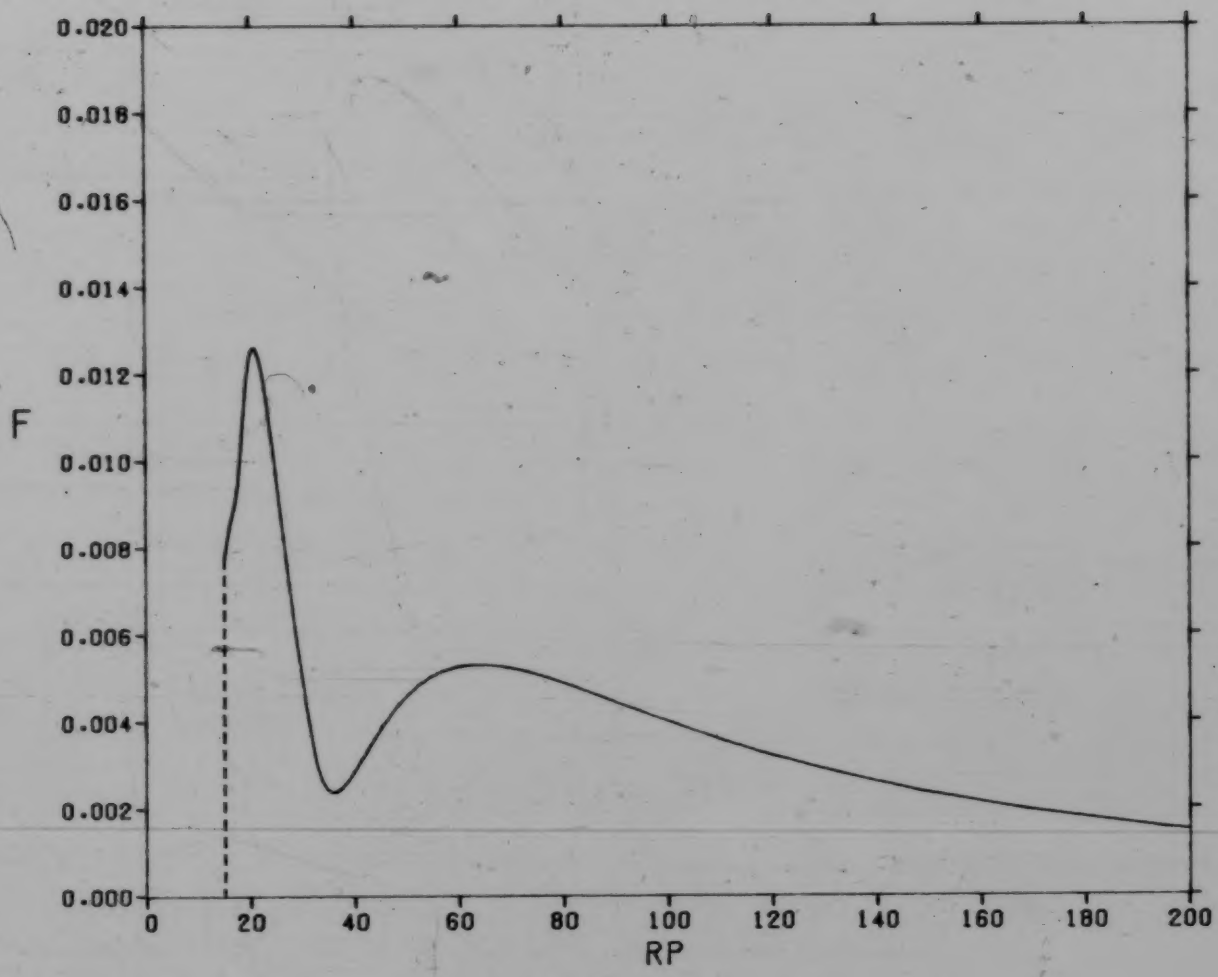
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 35

PLOT: #0021 10:31:01 MAY 5 1978 BY: SORP.Z1.F V1

WHEY PROTEIN POWDER

AIR-DRIED
48 HOURS, 25.0

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

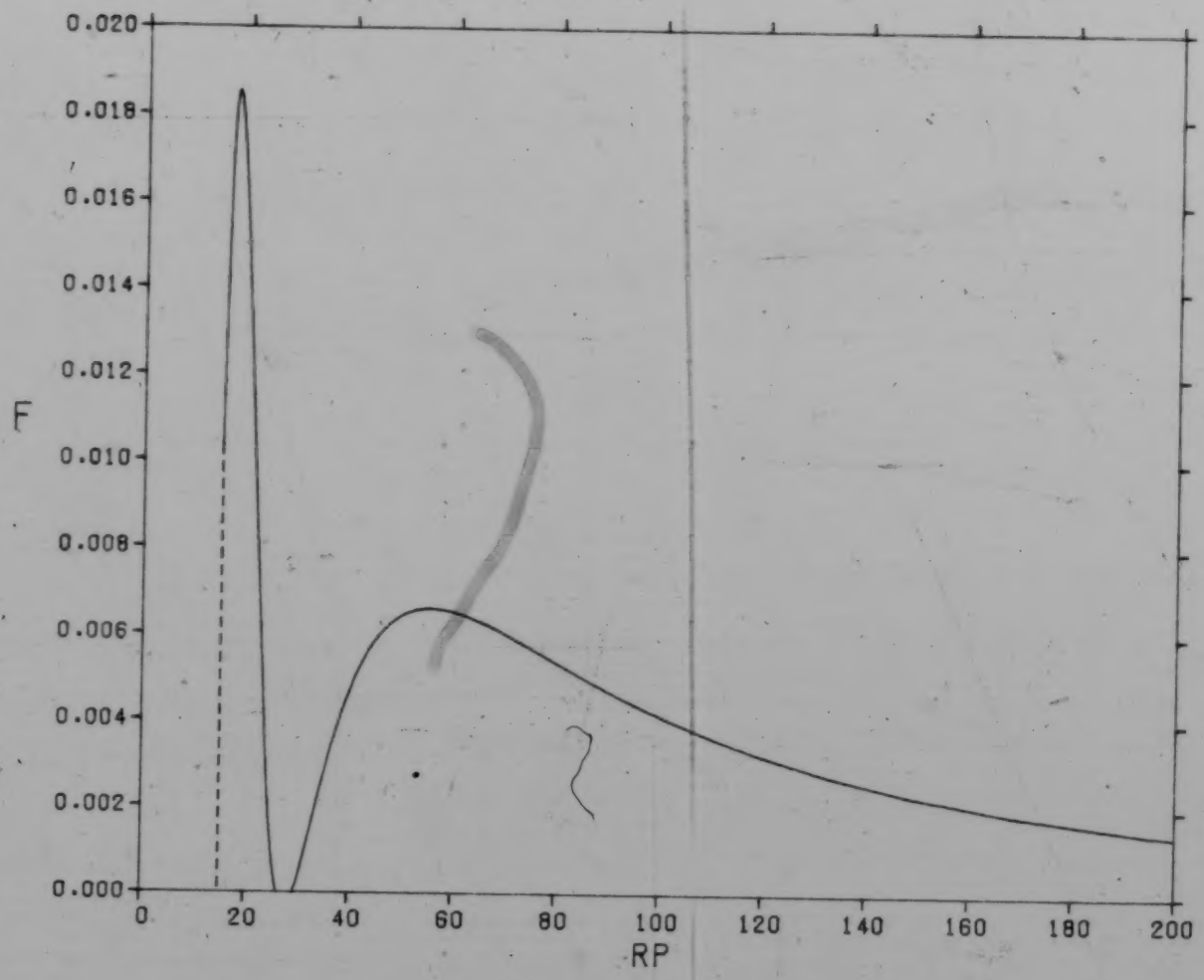
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 21

PLOT: *0001 09:28:40 APR 7 1978 BY: SORP.Z1.F V1

WHEY PROTEIN

HEAT PRECIPITATED VACUUM DRIED
36 HOURS. 60.0. 60 MESH.

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

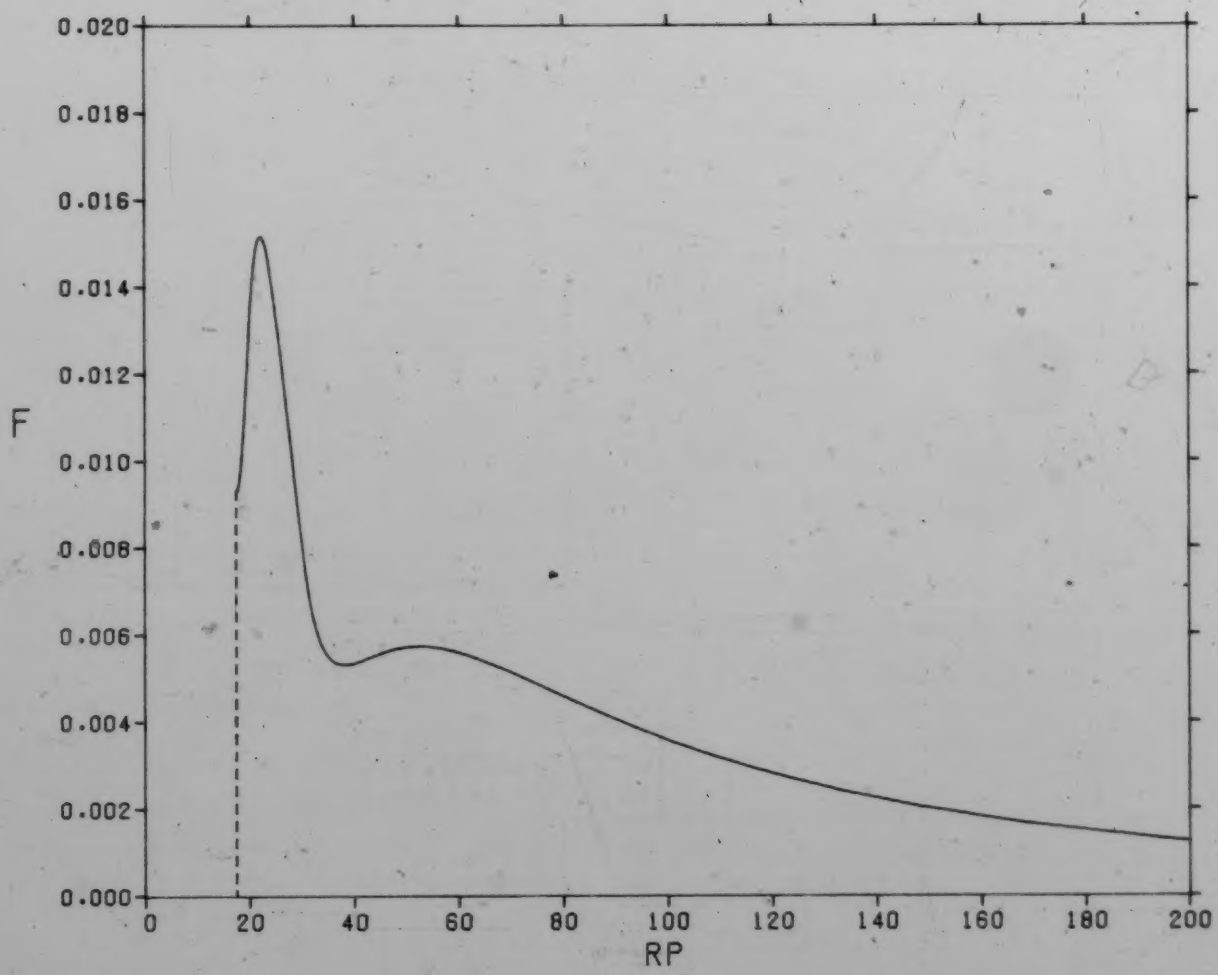
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, APR.-MAY., 1978
PAGE 43

PLOT: #0029 10:31:01 MAY 5 1978 BY: SORP.Z1.F V1

WHEY PROTEIN POWDER

DIALYSED, DENATURED
FREEZE-DRIED

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

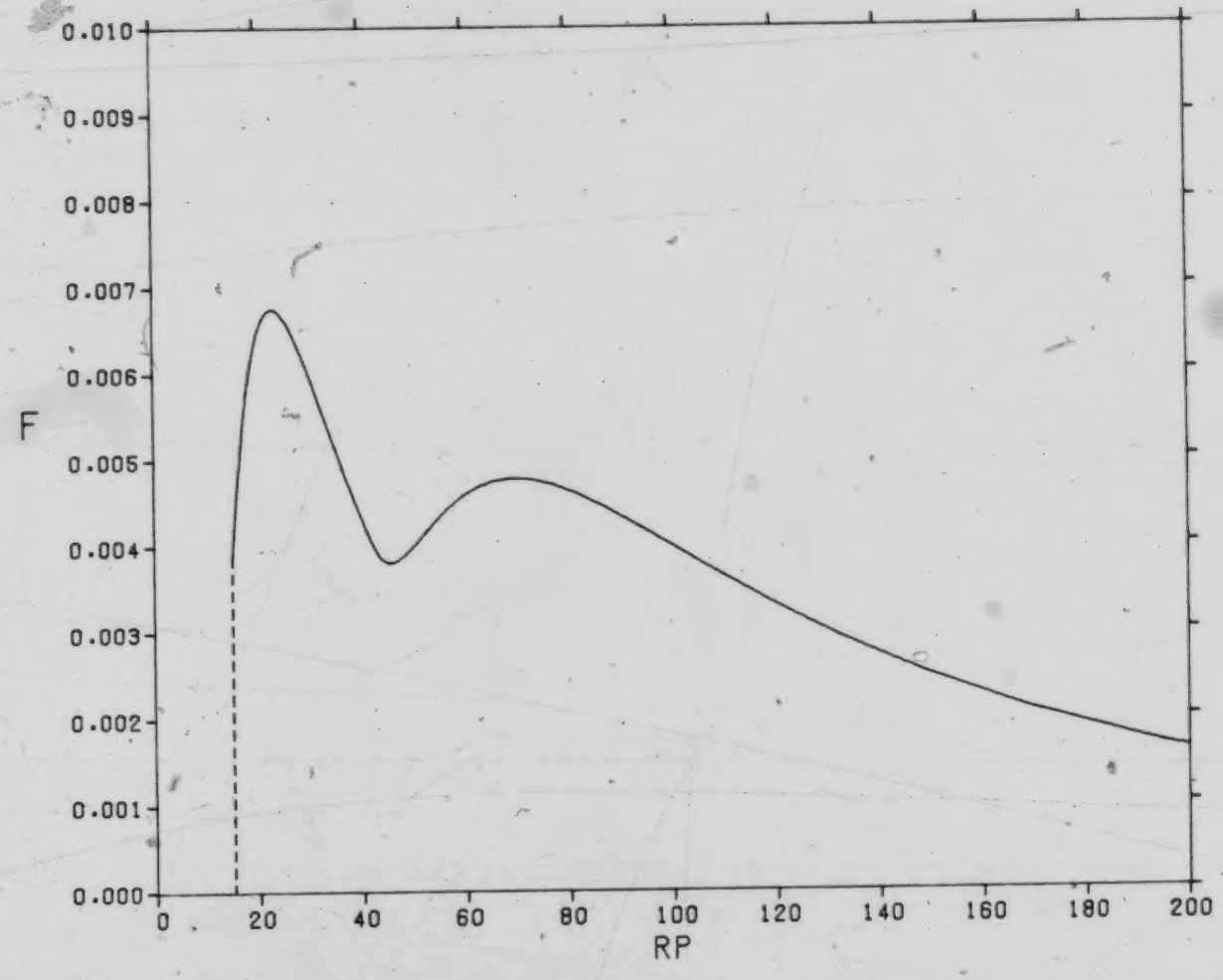
ADSORPTION DATA
ROBERT I. W. GREIG
LAB BOOK#1, JAN.-APR., 1978
PAGE 19

PLOT: #0005 09:28:40 APR 7 1978 BY: SORP.Z1.F V1

WHEY PROTEIN POWDER

DIALYSED. UNDENATURED
FREEZE-DRIED

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

ADSORPTION DATA
ROBERT I. W. GREIG
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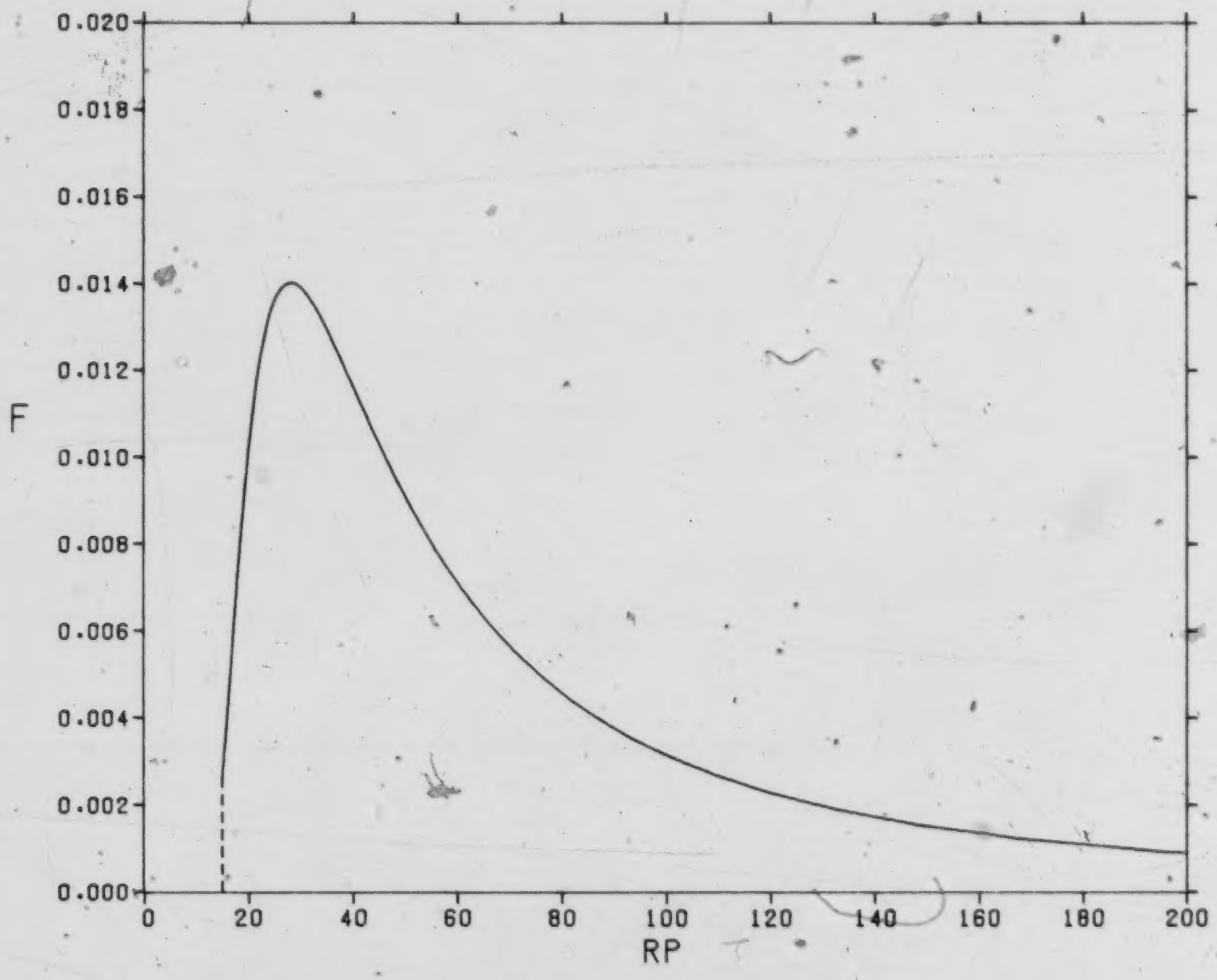
PLOT: #0009 09:28:40 APR 7 1978 BY: SORP.Z1.F V1.

WHEY PROTEIN CURD

HEAT-PRECIPITATED
WASHED. INITIAL MOISTURE 3.935G WATER/G SOLIDS

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

ADSORPTION DATA
ROBERT I. W. GREIG
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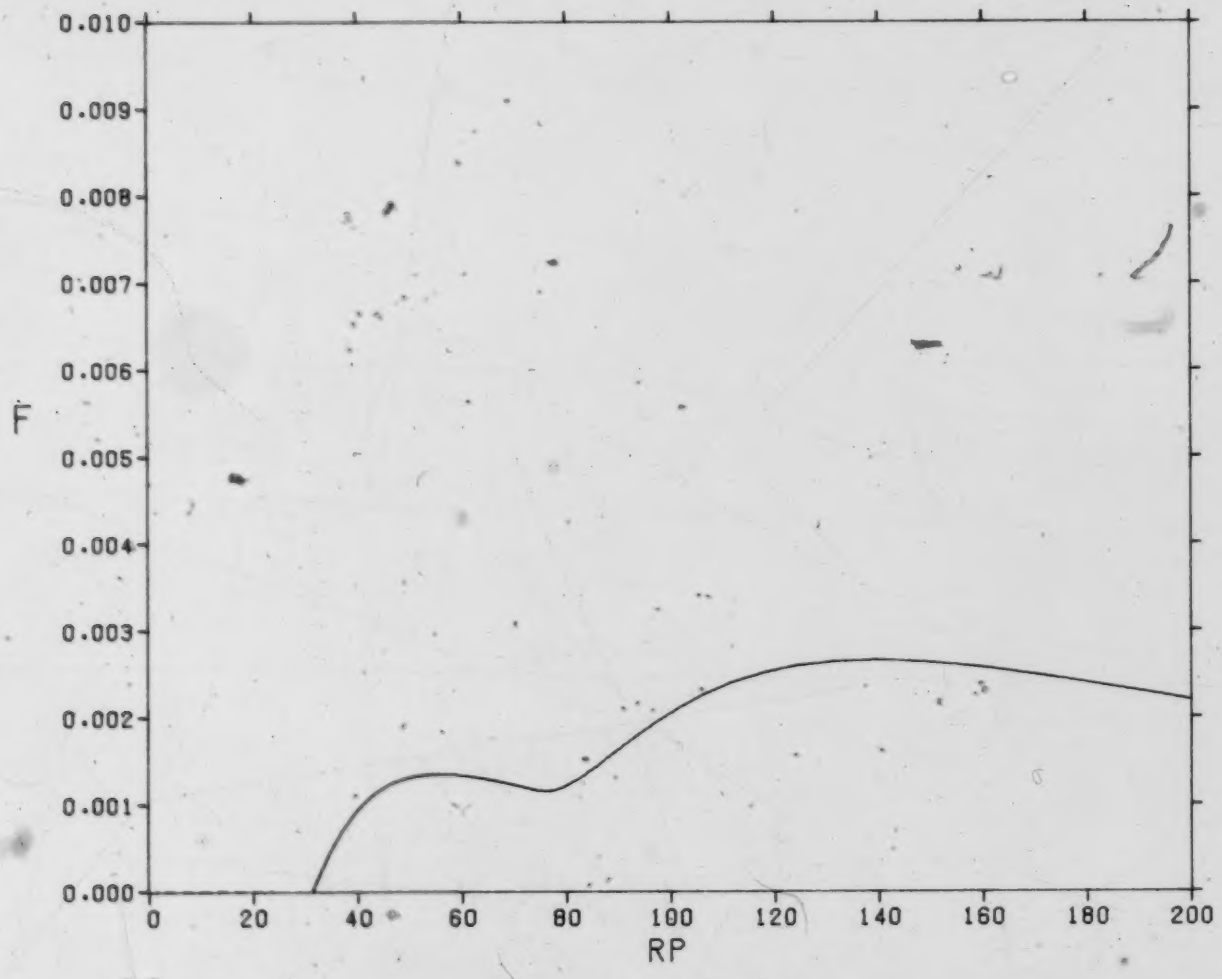
PLOT: =0025 09:28:40 APR 7 1978 BY: SORP.Z1.F V1

WHEY PROTEIN CURD

HEAT-PRECIPITATED
WASHED. INITIAL MOISTURE 3.935G WATER/G SOLIDS

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

DESORPTION DATA
ROBERT I. W. GREIG
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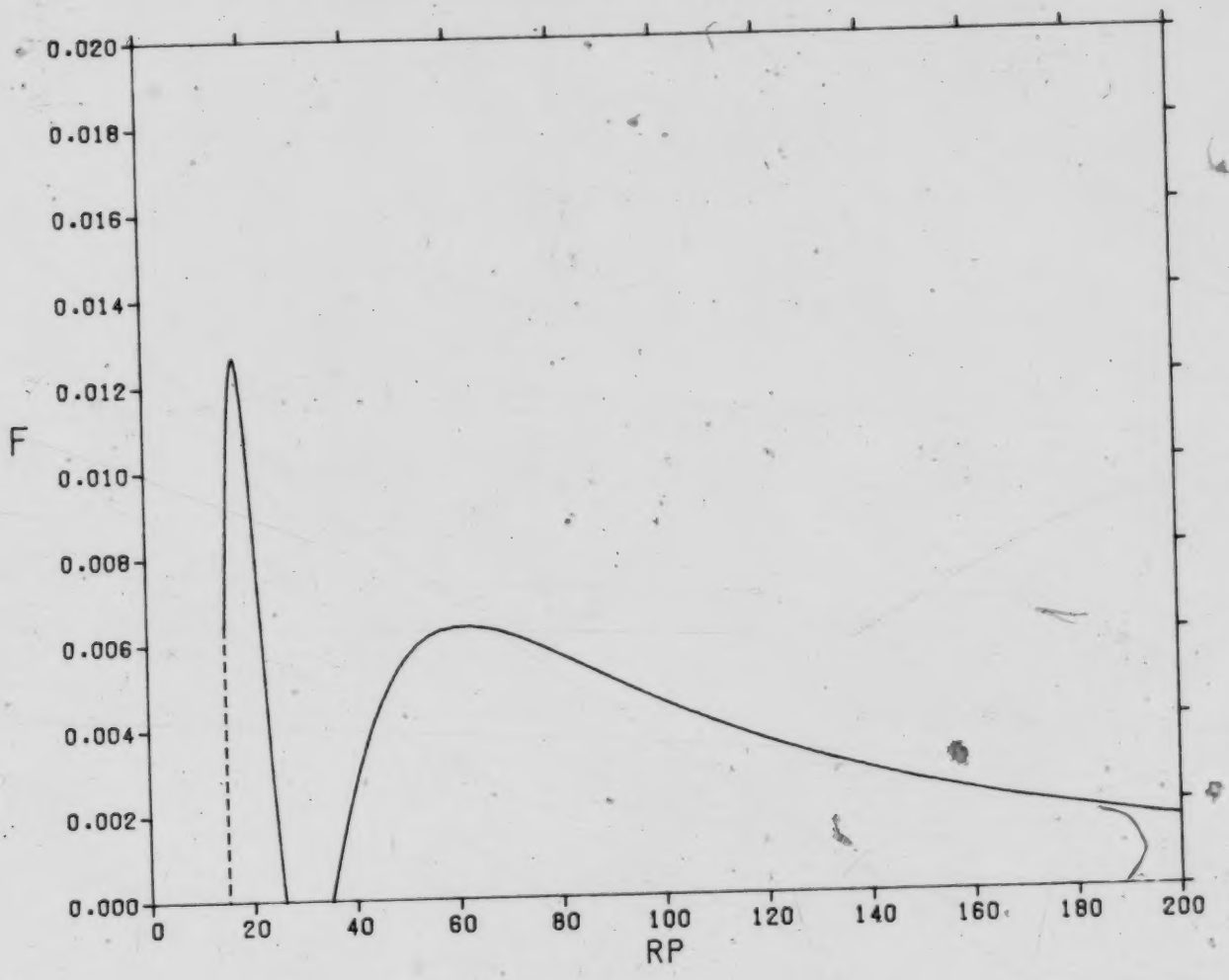
WHEY PROTEIN CURD

WASHED 3.935 G WATER / G SOLIDS READSORPTION

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

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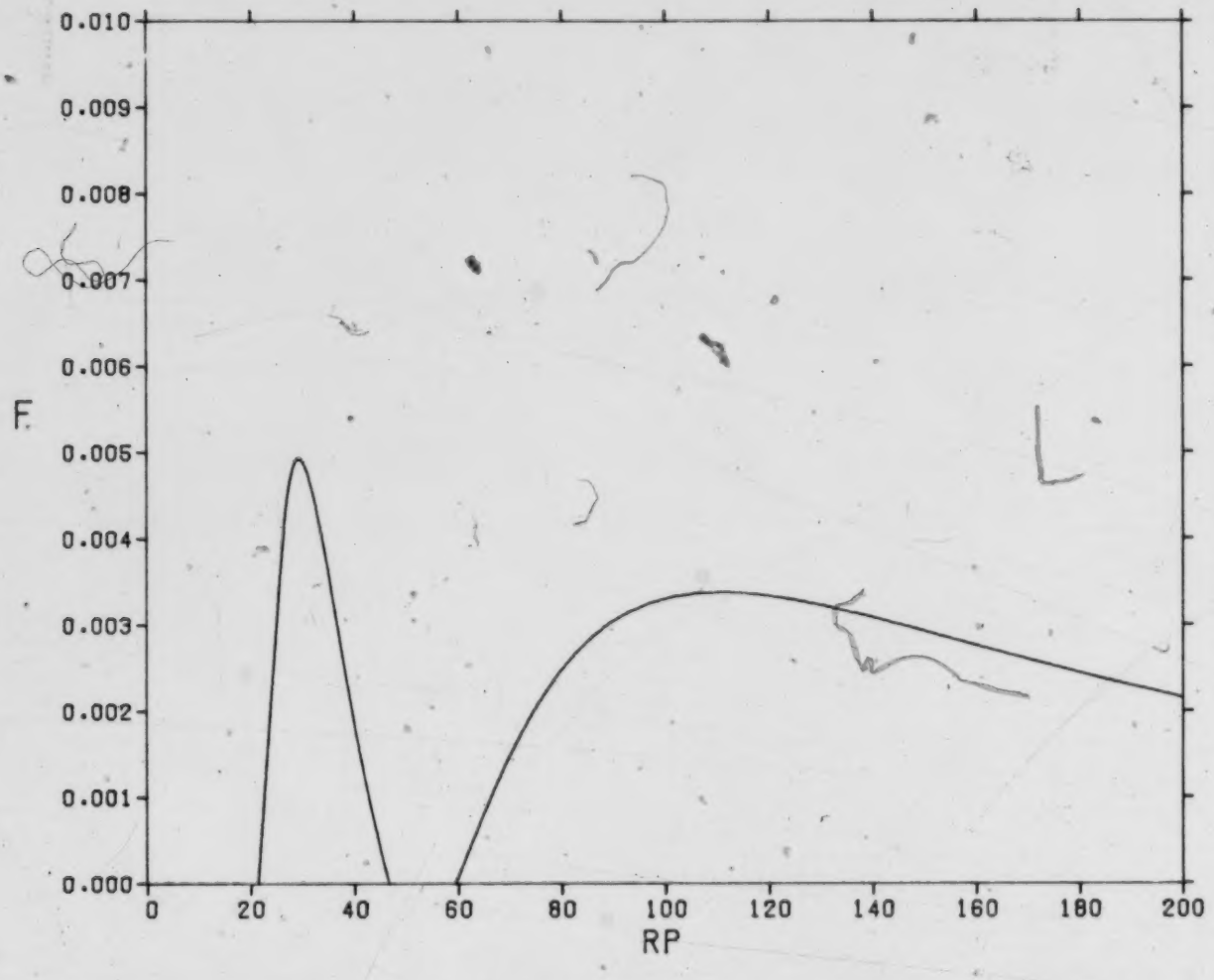
PLOT: #0005 10:31:01 MAY 5 1978 BY: SORP.Z1.F V1

WHEY PROTEIN CURD

WASHED 3.935 G WATER / G SOLIDS READSORPTION

TEMPERATURE 25.00 DEG. C



RP PORE RADIUS (ANGSTROMS)
F DENSITY FUNCTION

CYLINDRICAL PORE GEOMETRY

DESORPTION DATA
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APPENDIX E

ELECTRON MICROGRAPHS OF DENATURED WHEY PROTEIN POWDERS

The electron microscopy study (scanning and transmission) was carried out to see if differences in surface structure (if present) could be related to observed changes in the water holding and water sorption properties of the powders. Each powder (freeze-, spray-, air- and drum dried) was produced from batch 4.

Figures E.1, E.2, E.3 and E.4 clearly show that there were differences in the surface characteristics of the powders. Freeze dried and spray dried powders possessed large porous surface areas whereas the drum dried and air dried powders had relatively flat, non porous surfaces. Figures E.5 and E.6 show closer views of the air dried and spray dried powders. The differences in the surface characteristics (as viewed by SEM) correlate remarkably well with the BET monolayer values (section 5.3.3).

Figures E.7 and E.8 are TEM photomicrographs of freeze dried powder before and after hydration. These photographs show that the freeze dried powders were almost fully hydrated with a minimum number of unhydrated (or partially hydrated) protein aggregates. The drum dried (figures E.9 and E.10) and spray dried (figures E.11 and E.12) powders were only partially hydrated as shown by the large proportions of dense protein aggregates. The photomicrographs support the results obtained in the water holding study; freeze dried traditional lactalbumin had a much higher WHC than drum-, spray- or air dried traditional lactalbumin. The TEM photomicrographs for air dried traditional lactalbumin (not included in the appendix) were almost identical to those of the drum dried powder. The term "traditional lactalbumin" used above is synonymous with "denatured whey protein" for the purpose of this work.

It is interesting to note that the drum dried and spray dried powders (figures E.9 and E.11) had a "crust" around the surface of each particle. This "crust" was not observed in the hydrated powders. The appearance of the "crust" on the drum dried and spray dried powders could partially explain why these powders had lower water holding capacities than freeze dried powders (no "crust" observed).

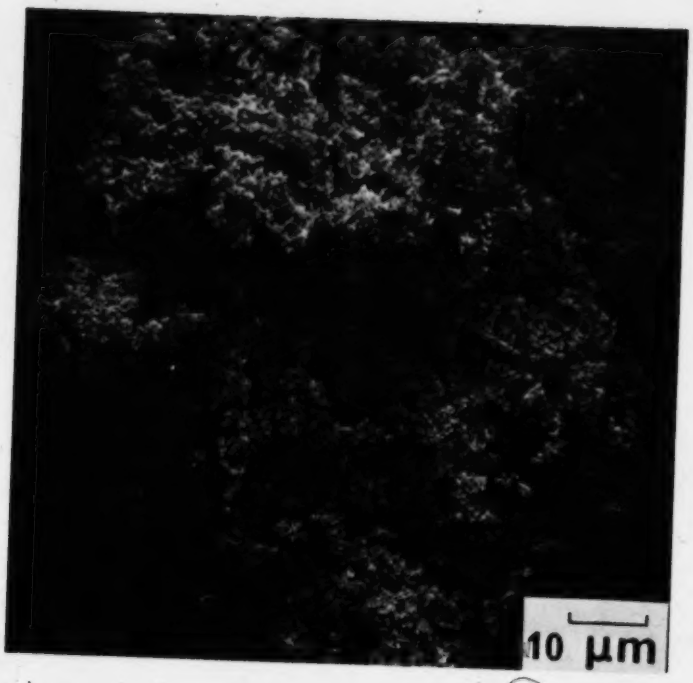

Figure E.1: SEM Photomicrograph, Freeze Dried Traditional Lactalbumin (x1140)

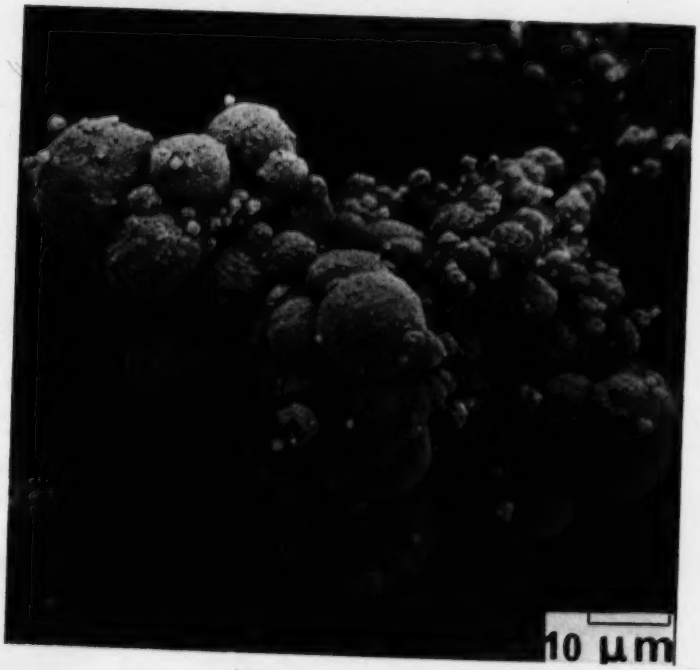

Figure E.2: SEM Photomicrograph, Spray Dried Traditional Lactalbumin (x1140)

Figure E.3: SEM Photomicrograph, Drum Dried Traditional Lactalbumin (x114)

Figure E.4: SEM Photomicrograph, Air Dried Traditional Lactalbumin (x57)

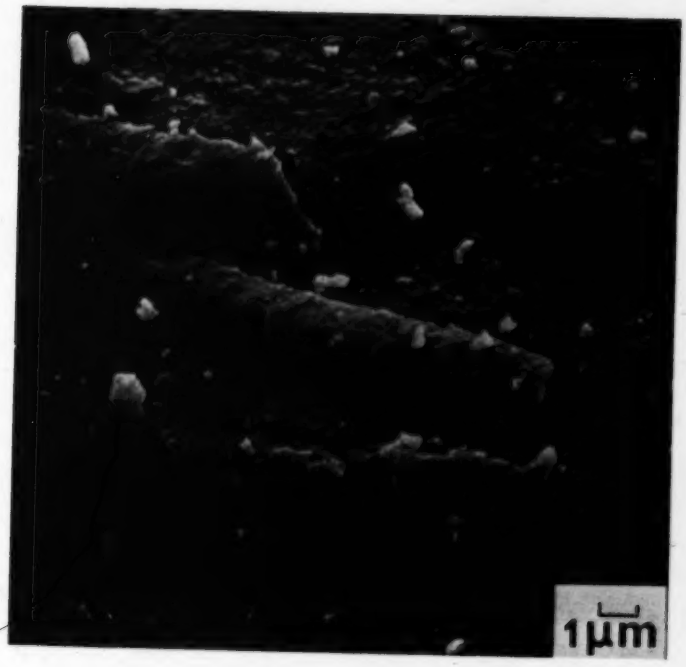

Figure E.5: SEM Photomicrograph, Air Dried Traditional Lactalbumin (x5700)

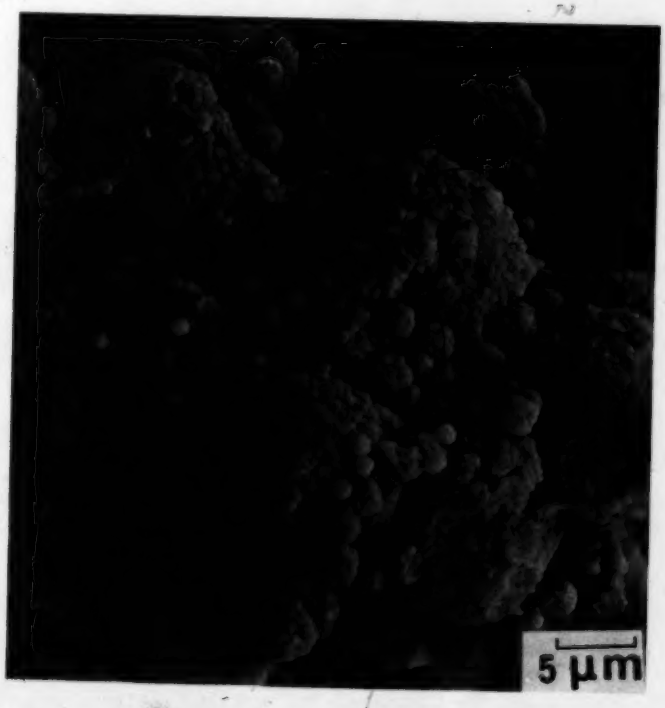

Figure E.6: SEM Photomicrograph, Spray Dried Traditional Lactalbumin (x2280)

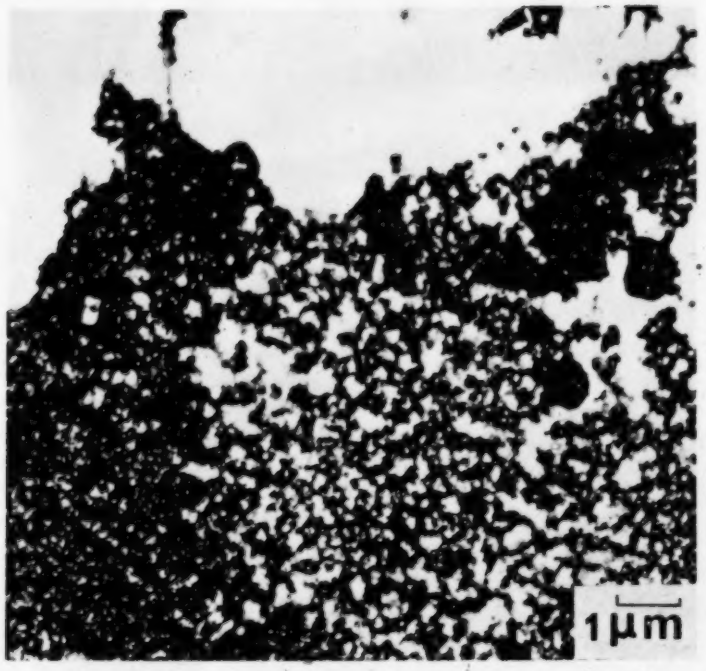

Figure E.7: TEM Photomicrograph, Freeze Dried Traditional Lactalbumin (x8925)

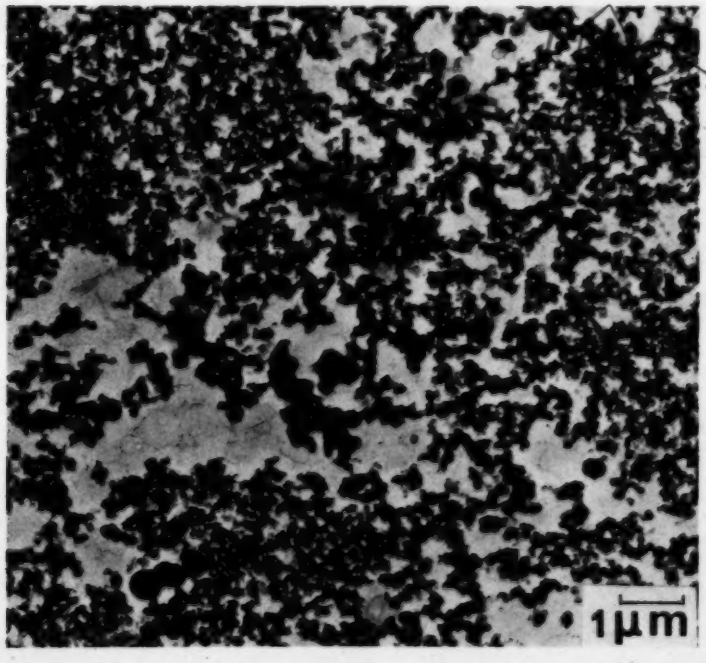

Figure E.8: TEM Photomicrograph, Freeze Dried (hydrated) Traditional Lactalbumin (x8925)

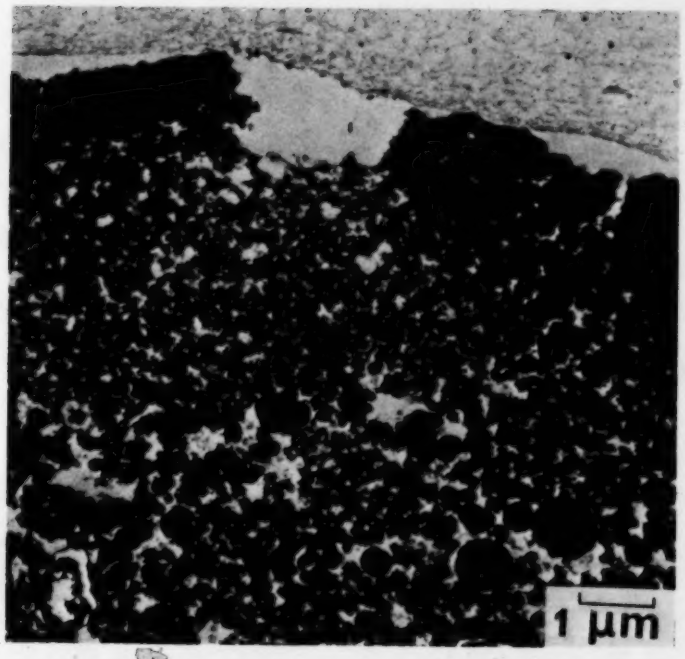

Figure E.9: TEM Photomicrograph, Drum Dried Traditional Laetalbumin (x11063)

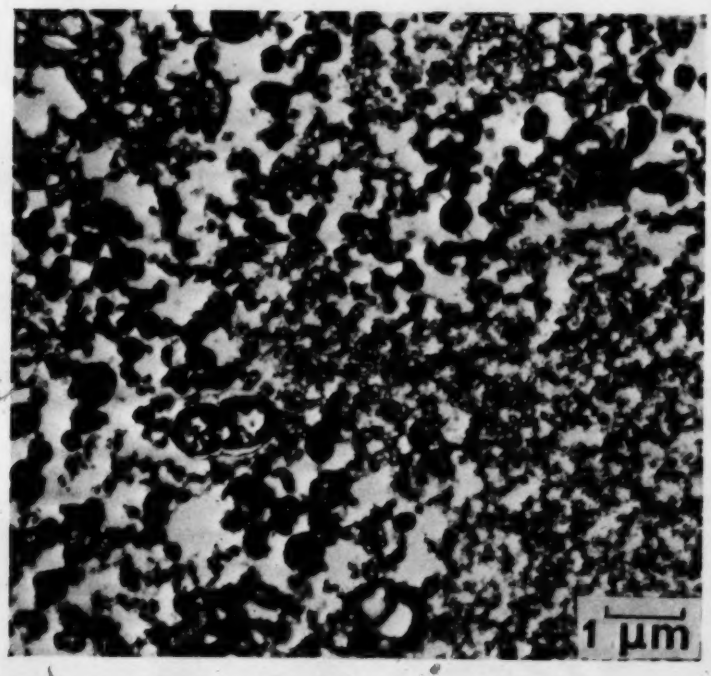

Figure E.10: TEM Photomicrograph, Drum Dried (hydrated) Traditional Lactalbumin (x11063)

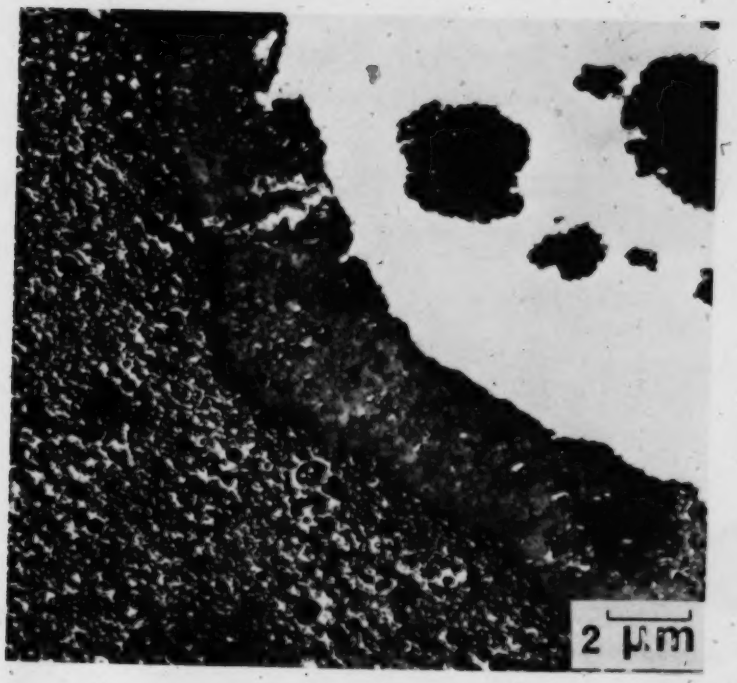

Figure E.11: TEM Photomicrograph, Spray Dried Traditional Lactalbumin (x5963)

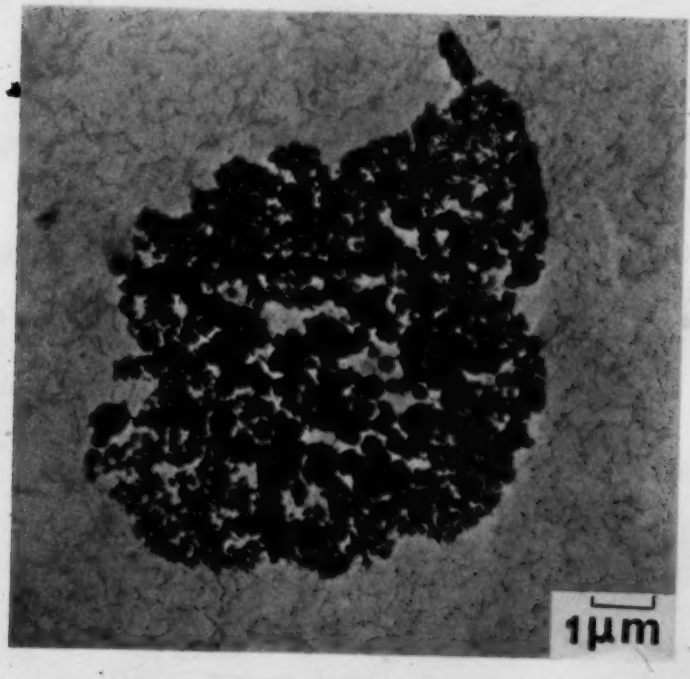

Figure E.12: TEM Photomicrograph, Spray Dried (hydrated) Traditional Lactalbumin (x8925)